

RICKETS IN VERY LOW-BIRTH-WEIGHT INFANTS

BORN AT BARAGWANATH HOSPITAL

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A Dissertation Submitted to the Faculty of  
Medicine, University of the Witwatersrand, Johannesburg  
for the degree of Master of Medicine. Johannesburg 1991.

## ABSTRACT

Disturbed mineral and bone metabolism is frequently found in very low-birth-weight infants fed breast-milk during the first three months of life. The study was designed to assess the prevalence of this disturbed mineral homeostasis in a very low-birth-weight population at Baragwanath Hospital and to determine whether the addition of a preterm infant formula to the feeds reduced the prevalence and increased the rate of weight gain. Fifty three neonates weighing less than 1200g born at Baragwanath Hospital were monitored for weight gain, growth and for biochemical and radiological evidence of metabolic bone disease. The infants were randomized to receive either breast-milk only feeds or a combination of breast-milk and a premature formula in order to assess the effect of the different feeds on the development of bone disease. Weight gain and growth were similar in both groups. Calcium and phosphorus intakes were higher in the mixed feeding group. However, serum calcium and phosphorus values were similar in the two groups throughout the study. The breast-milk group had significantly higher alkaline phosphatase levels. Radiological rickets was uncommon in both groups, although periosteal reactions and osteopenia occurred frequently and with similar prevalence in both groups. Overt rickets is not a major problem in very-low-birth-weight infants born at Baragwanath Hospital, although raised serum alkaline phosphatase values occur frequently. Feeding with breast-milk and a premature infant formula in

equal proportions (as opposed to breast-milk only) does not appear to have any effect on weight gain and growth in very low-birth-weight infants, but does partially prevent the pathological rise in alkaline phosphatase levels.



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## PREFACE

Metabolic bone disease is a well recognised problem in low-birth-weight infants, the reported incidence varying from 13 to 32%. It is more prevalent in those infants born weighing less than 1200g and the supplementation of calcium and phosphate to the milk feeds of very low-birth-weight infants has been shown to decrease the prevalence of metabolic bone disease. In view of Baragwanath Hospital policy of encouraging the use of breast feeding where possible as well as the lack of information on the prevalence of this problem in these very small infants at Baragwanath Hospital, it was decided to study the prevalence of rickets in infants under 1200g on the basis of biochemical and radiological evidence as well as assessing the effect of a premature infant formula on the prevalence.

I am greatly indebted to Professor John M. Pettifor for his guidance, assistance and encouragement throughout the duration of this project. I would also like to thank Mr. Gopal P. Moodley, Mrs. Meropi Cavaleros and Mrs. Diane Zachen for doing the laboratory investigations. I would also like to express my appreciation to my mother, colleagues and friends for their support and to all those who helped in the face of disaster.

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## CHAPTER 1

### INTRODUCTION

Metabolic bone disease is a collective term indicative of a generalized disturbance of bone homeostasis, generally resulting from an abnormality in the factors influencing bone formation and/or mineralization and bone resorption.

In the very low-birth-weight (VLBW) infant the disease ranges from mild biochemical abnormalities with no radiological abnormalities, through mild under-mineralization to severe rickets with fractures (1, 2).

The pathogenesis of the bone disease has been attributed to a number of factors including deficiency of calcium, phosphorus and/or vitamin D (1, 3, 4).

In this chapter, I shall briefly discuss normal bone metabolism and mineralization as well as the factors that affect these processes, prior to reviewing in detail previous work on metabolic bone disease in VLBW infants.

### 1. BONE AND THE VITAMIN D ENDOCRINE SYSTEM

#### 1.1. BONE

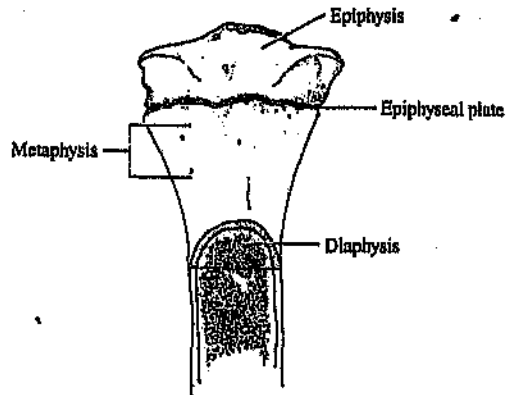
Bone is a composite of a hard, crystalline mineral and a microfilbrillar matrix into which mineral is incorporated. Prior to mineralization, bone matrix which is composed

largely of collagen fibres, is deposited in lamellae. Mineralization of this matrix then occurs. This mineral is mostly in the form of a complex salt that is regarded as a hydroxyapatite with the basic formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . Nearly 90% of calcium and phosphorus ions resides in these hydroxyapatite crystals. Bone tissue, thus provides an enormous reservoir of both calcium and phosphorus that can function to stabilize the concentrations of these ions in extracellular fluid. Bone mineralization is in turn directly dependent on the concentration of calcium and phosphate at the site of mineralization. It is also indirectly dependent on the circulating concentrations of calcium and phosphorus regulating hormones and nutritional factors (Table 1). The endocrine effects on bone mineralization will be discussed in greater detail in section 1.2.

**TABLE 1.** Factors affecting bone mineralization

<b>Required at bone site</b> Adequate concentration of calcium and phosphate Normal pH	
<b>Endocrine effects on mineralization</b> 1. Increases bone resorption <ul style="list-style-type: none"> <li>a. ↑ increasing osteoclast formation <ul style="list-style-type: none"> <li>- PTH</li> <li>- 1,25 dihydroxyvitamin D</li> <li>- thyroxine</li> <li>- growth hormone</li> </ul> </li> </ul> 2. Decreases bone resorption <ul style="list-style-type: none"> <li>a. By decreasing osteoclast formation <ul style="list-style-type: none"> <li>- calcitonin</li> <li>- glucocorticoids</li> <li>- oestrogens</li> </ul> </li> <li>b. By increasing osteoblast formation <ul style="list-style-type: none"> <li>- calcitonin</li> <li>- oestrogens</li> <li>- growth hormone</li> </ul> </li> </ul>	
<b>Nutritional effects on mineralization</b> 1. Required for adequate mineralization <ul style="list-style-type: none"> <li>a. vitamin D</li> <li>b. calcium and phosphorus</li> <li>c. magnesium</li> <li>d. zinc</li> <li>e. copper</li> </ul> 2. Inhibitors of mineralization <ul style="list-style-type: none"> <li>a. magnesium</li> <li>b. aluminium</li> <li>c. fluoride</li> </ul>	

Bone is also a growing living tissue which undergoes growth and continual remodelling in order for it to provide mechanical support and protection. In the growing infant and child, the epiphyses are separated from the diaphyses by a cartilaginous growth plate and linear growth of tubular bones occurs at this epiphyseal growth plate (Figure 1).



**FIGURE 1.** Schematic representation of bone illustrating different regions.

The initial step in bone mineralization is calcification of the cartilage at the metaphyseal side of the epiphyseal plate forming the provisional zone of calcification. The formation of new bone at the metaphysis is then preceded by the resorption of the calcified cartilage and reprecipitation of mineral in the new matrix laid down by osteoblasts that line the invading capillaries.

Linear bone growth ceases after epiphyseal closure. Continual remodelling of bone occurs primarily at the trabecular bone surface. Resorption of bone mineral by osteoclasts is followed by new matrix formation and mineralization of this matrix.

## 1.2. CALCIUM AND PHOSPHORUS HOMEOSTASIS

### 1.2.1. Hormones of calcium and phosphorus homeostasis

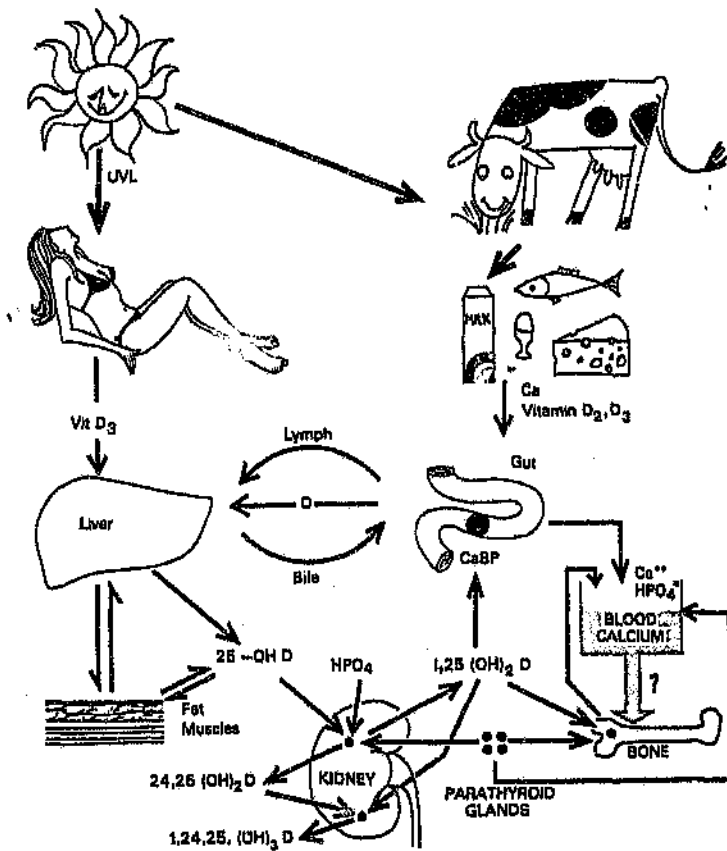
Calcium and phosphorus are essential to a great number of cellular functions, thus the extracellular levels of these ions (in particular calcium) are finely controlled. The maintenance of calcium and phosphorus homeostasis involves the delicate inter-relationships of absorption by the intestine, accretion and resorption by bone tissue and by filtration and reabsorption by the kidney. The three principal hormones involved in calcium and phosphorus homeostasis are vitamin D, parathormone (PTH) and calcitonin. (Table 2).

**TABLE 2.** Actions of vitamin D, parathormone and calcitonin in calcium and phosphorus homeostasis.

EFFECT	CALCITONIN	PTH	VITAMIN D
<b>INTESTINAL</b>			
Calcium absorption	↓?	→	↑
Phosphate absorption	?	→	↑
<b>RENAL</b>			
Phosphate excretion	↑	↑	↓
Calcium excretion	↑	↓	↓
Hydrogen excretion	→	↓	?
Potassium excretion	Slight ↑	↑	?
Sodium excretion	↑	↑	?
Adenyl cyclase activity	→	↑	?
<b>SKELETAL</b>			
Calcium mobilization	↓	↑	↑
Mineralization of bone matrix			↑
<b>OTHER</b>			
Plasma levels of calcium	↓	↑	↑
Plasma levels of phosphate	↓	↓	?↑
Body weight	?	?	↑

### 1.2.1.1. Vitamin D physiology

The physiology of vitamin D (vit D) has been extensively reviewed (5, 6). The metabolic conversions undergone by vitamin D in order to fulfil its hormonal functions are depicted in Figure 2.



**Figure 2.** Schematic overview of vitamin D - its sources, activation and homeostatic control.

Vitamin D requirements are met from a combination of two sources - sunlight and dietary intake. The first proceeds in the skin where, upon exposure to ultraviolet irradiation (290-320nm), 7-dehydrocholesterol is first photochemically converted to precholecalciferol (previtamin D) in the stratum Malpighii. This is then converted more slowly by molecular re-arrangement into vitamin D<sub>3</sub> (cholecalciferol) (Figure 3) and released into the circulation over 2 - 3 days. This pathway is the natural source of antirachitic activity when exposure to sunlight is adequate.

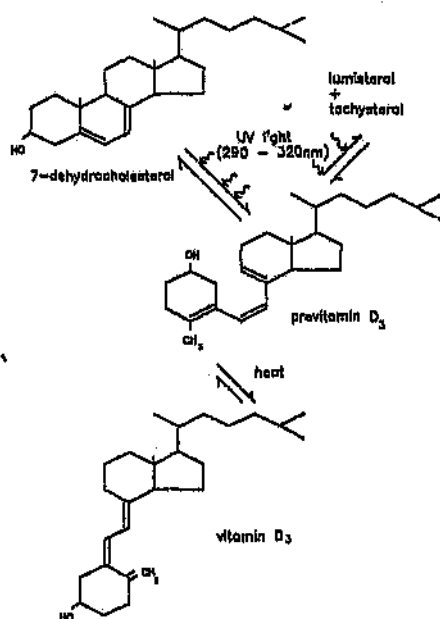


Figure 3. The photosynthetic conversion of 7-dehydrocholesterol by sunlight to vitamin D<sub>3</sub>.



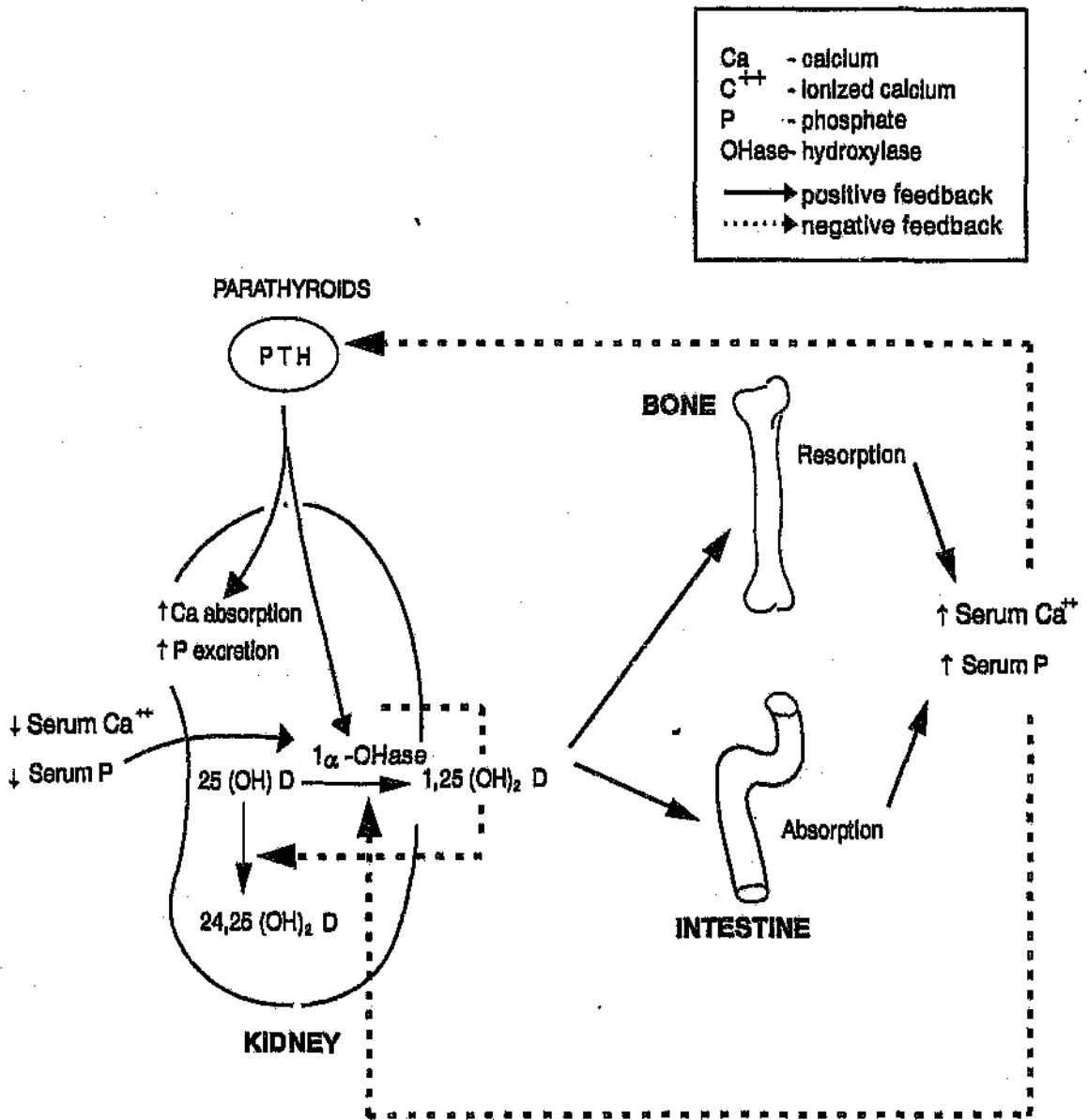
The second important source of vitamin D available to man is ergocalciferol (vitamin D<sub>2</sub>) which arises from ultraviolet irradiation of ergosterol present in plants and is generally known as dietary vitamin D. The main dietary sources of vitamin D<sub>2</sub> are fortified dairy products and fish liver oil. On a weight basis, 25ng of vitamin D<sub>2</sub> has been designated as 1 international unit (IU) of antirachitic activity. The recommended daily allowance of vitamin D for children is 400IU, although dietary requirements depend on the amount of vitamin D formed in the skin under ultraviolet irradiation.

Vitamin D<sub>3</sub> (endogenous) and vitamin D<sub>2</sub> (exogenous) are transported in the plasma bound to a specific alpha-2-globulin known as vitamin D binding protein (DBP). Vitamin D absorbed from the gastro-intestinal tract is transported in chylomicrons in the lymphatics.

Depending on the vitamin D status of the individual, vitamin D is either deposited in the body stores or taken up by the liver and converted to 25-hydroxyvitamin D (25-OHD). 25-OHD is the major circulating form of vitamin D and is transported in the blood bound to DBP to the kidney. There it is converted to the active metabolite, 1,25-dihydroxyvitamin D (1,25-(OH)<sub>2</sub>D). A further metabolite, 24,25-dihydroxyvitamin D, is also formed in the kidney along with several other

relatively inactive metabolites.

The formation of 25-OHD does not appear to be strictly controlled, but the formation of  $1,25-(OH)_2D$  is regulated in a feedback fashion by plasma calcium and phosphorus concentrations (Figure 4).



**Figure 4 :** Feedback control of the formation of the active vitamin D metabolite 1,25 dihydroxyvitamin D.

#### 1.2.1.2. Parathyroid hormone

Parathyroid hormone (PTH) is secreted by the parathyroid glands. Its action is to increase the plasma concentration of ionized calcium by directly mobilizing calcium from bone, and increasing renal tubular reabsorption of calcium. It also increases the formation of 1,25 dihydroxyvitamin D, the physiologically active metabolite of vitamin D which controls the intestinal transport of calcium and phosphate. PTH also depresses the plasma phosphate by decreasing renal tubular phosphate reabsorption (phosphaturic action).

The major controlling factor in determining the rate of PTH synthesis and release is the concentration of calcium ions in the fluid perfusing the parathyroid gland. When the plasma ionized calcium level is high, secretion of PTH is inhibited and calcium is deposited in the bones. When serum ionized calcium is low, the reverse occurs. Elevated plasma phosphate levels stimulate PTH secretion, but only through their secondary effect of lowering the plasma ionized calcium concentrations.

#### 1.2.1.3. Calcitonin

Calcitonin is a calcium lowering hormone that is secreted primarily by the parafollicular cells of the thyroid gland. It acts by inhibiting bone resorption. Its role in normal

bone physiology is probably minimal.

The effects of these calcitropic hormones are summarized in Figure 5.

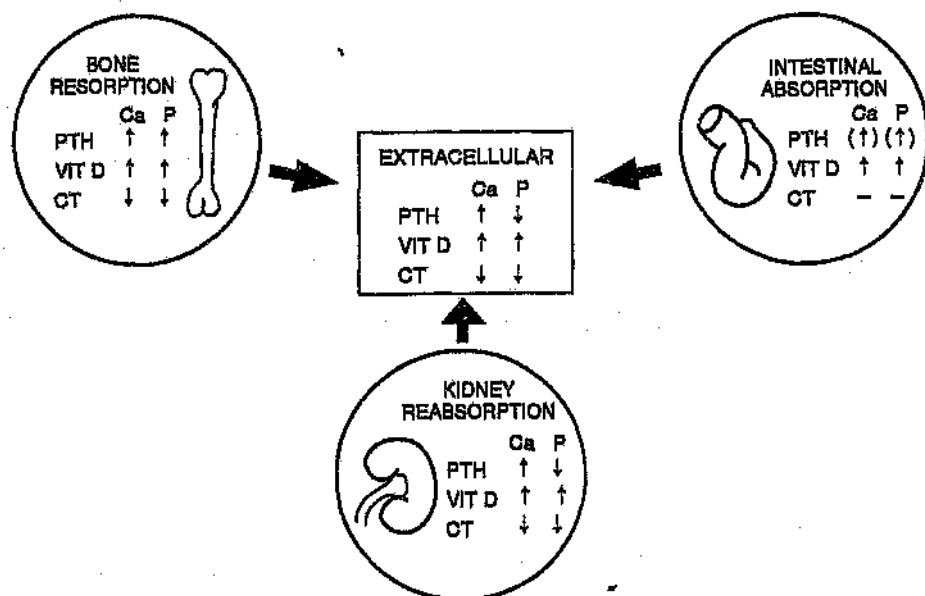


Figure 5. Calcitropic hormones and their actions on target organs and effects on serum calcium and phosphorus.

### 1.2.2. Calcium homeostasis

The adult human body contains about 1100g of calcium, mostly found in the skeletal system as hydroxyapatite (as discussed in section 1.1). The plasma calcium, normally about 2.5mmol/l, is partly protein bound and partly ionized (Table 3).

**TABLE 3.** Distribution of calcium (mmol/l) in normal human plasma.

<b>Diffusable</b>		<b>1.34</b>
Ionized calcium	1.18	
Complexed to $\text{HCO}_3^-$ , citrate, etc.	0.16	
<b>Nondiffusable (protein-bound)</b>		<b>1.16</b>
Bound to albumin	0.92	
Bound to globulin	0.24	
<b>Total plasma calcium</b>		<b>2.50</b>

The physiologically important fraction is the ionized fraction, which plays a role in essential cellular processes such as coagulation, normal cardiac function and skeletal muscle and nerve function. It is, therefore, essential that ionized calcium is finely controlled.

Calcium absorption from the gut (primarily in the duodenum and jejunum) is controlled by the body's need for calcium and thus all the calcium ingested is not necessarily absorbed. In adults intestinal calcium absorption is both active and passive. The former is saturable and greater for individuals previously adapted to low calcium intake. Passive transport is directly proportional to intraluminal calcium concentration.

Intestinal calcium absorption is controlled largely by the concentration of 1,25-dihydroxyvitamin D (Figure 6). Low serum calcium also stimulates PTH secretion which stimulates

1,25-dihydroxyvitamin D formation in the kidney and thus increases calcium absorption. Increased calcium absorption during active growth, pregnancy and lactation in order to meet increased calcium requirements is mediated not only through PTH, but also by the action of growth hormone, oestrogens and prolactin through their control of 1,25-dihydroxyvitamin D production.

Calcium absorption is decreased by cortisol and other steroids with glucocorticoid properties, malabsorptive states (particularly fat malabsorption) and the presence of large amounts of phosphate or phytate in the gut.

In the event that dietary intake or availability of calcium is decreased, the regulatory role of bone and kidney come into play to maintain a constant serum calcium level. Thus, serum calcium may become elevated through stimulation of bone resorption or through PTH stimulation of tubular reabsorption of calcium in the kidney. Concomitantly, PTH stimulates phosphate excretion, so that as serum calcium concentration increases, there is usually a fall in plasma phosphate level.

On a daily basis, the renal glomeruli of an adult filter some 10000mg calcium, but renal tubular reabsorption is so efficient that under normal circumstances only 100-150mg appear in the urine. In the event of hypercalcaemia, the urinary excretion of calcium rises in a compensatory fashion.

The renal tubular reabsorption of calcium is stimulated by PTH. Increased urinary excretion of calcium is stimulated by phosphate deprivation, acidosis, adrenal steroids and saline diuresis.

### 1.2.3. Phosphate homeostasis

Under normal circumstances, inorganic phosphate is present in plasma of an adult at a level of approximately 1.2mmol/l (2.5 - 4.3mg/dl) in adults. Approximately 10% of this is protein bound. The remainder exists as free phosphate, either  $\text{HPO}_4^{2-}$  or  $\text{H}_2\text{PO}_4^-$ . Phosphate is also required as organic phosphate in which it functions in enzymatic and structural proteins.

Phosphate in the body is partitioned among 3 major pools: the kidney ultrafiltrate, the readily exchangeable bone fraction and the intracellular compartments of soft tissues. The dynamics of phosphate metabolism are not particularly different from those of calcium. Under normal circumstances, approximately 70% of dietary phosphate is absorbed. This is not as closely controlled as calcium absorption and plays a relatively minor role in phosphate homeostasis. Absorption of phosphate is interrelated in a complex fashion with the presence of calcium and can be stimulated by a low calcium diet as well as by vitamin D or its metabolites (Figure 5). The intestinal absorption of phosphate is inhibited by high dietary calcium levels, aluminium hydroxide ingestion and other divalent cations with which it forms insoluble



phosphates.

Phosphate is excreted mainly by the kidney, this being the major controlling mechanism of serum phosphate levels. The control of renal excretion of phosphate can be explained by a reabsorptive tubular maximum function (TmP). When the quantity of phosphate filtered decreases below the maximum reabsorptive capacity, tubular reabsorption of phosphate becomes more complete. The handling of phosphate by the kidney is determined by the glomerular filtration rate (GFR), tubular reabsorption and possibly a minor role by tubular secretion. The equilibrium concentration (TmP/GFR) is used as a measure of tubular reabsorption of phosphate in relation to renal function. This equilibrium concentration is influenced by age and maturity. This explains the variation in serum phosphate with age (Table 4). The high TmP/GFR of the infant and child with normal vitamin D status during the growth period maintains high concentrations of serum phosphate during this period. This is obviously important in the rapid mineralization of cartilage and bone.

TABLE 4. Changes in serum phosphate with age.

AGE	SERUM Pi (mmol/l)
Premature infants	2.5
Full term infants - first week	2.0
1 to 10 years	1.5
Adult	1.2

As already indicated, the major endocrine function of PTH is to regulate the tubular reabsorption of phosphate. Thus high circulating levels of PTH stimulate the urinary excretion of phosphate by blocking tubular reabsorption of inorganic ion present in glomerular filtrate (Figure 5). Other hormones such as calcitonin, thyroid hormones and growth hormone, probably through the action of insulin growth factor-1, also influence the renal handling of phosphate.

#### 1.2.4. CONCLUSIONS

A complex inter-relationships exist between bone, calcium, phosphate and the various hormones controlling these processes. Normal vitamin D status and adequate calcium and phosphate supplies are required for adequate bone mineralization. Bone, in turn, provides a reservoir for both calcium and phosphate with movement of these ions into and out of bone.

### 1.3. CALCIUM, PHOSPHORUS AND VITAMIN D METABOLISM IN THE PREMATURE NEONATE

The needs of the premature neonate differ from those of the term neonate, infant and adult as the premature neonate has a faster growth rate, has many physiological functions that are immature and has also not had the benefit of completing gestation in an optimum intra-uterine environment. Therefore, it is important to consider the calcium, phosphorus and vitamin D requirements and metabolism of the premature infant specifically. I will discuss these requirements in the newborn premature infant and then discuss the diagnosis, pathogenesis and prevention of metabolic bone disease in this group of neonates. .

#### 1.3.1. Calcium and phosphorus

In the foetus, 80% of the calcium and phosphorus accretion required for bone mineralization occurs during the third trimester (7). The foetus at 25-36 weeks grows at a rate of approximately 25g/day and accumulates 100-120mg/kg/day calcium and 60-75mg/kg/day phosphorus (Table 5) (8). These accretion rates are the highest that an individual will attain throughout life. Although calcium and phosphorus metabolism are linked, the handling of these minerals in the premature neonate will be discussed separately.

**TABLE 5.** Daily increments of body weight, calcium and phosphorous in the foetus.

Gestational age (weeks)	Weight (g)	Calcium (mg)	Phosphorus (mg)
24-25	11.4	61	39
28-29	22.6	129	83
32-33	27.1	193	123
36-37	35.7	341	212
39-40	17.1	302	179

### 1.3.2. Calcium in the premature neonate

1.3.2.1. Requirements. Calcium requirements in utero are met by active calcium transport across the placenta. Maternal adjustments to the calcium drain have been extensively investigated and reviewed (9, 10), but are not relevant to the current discussion. Following premature birth and the loss of maternal supplies, calcium requirements must be met by external sources. In order to establish the nutritional requirements of the premature infant, Ziegler et al (8) have used the factorial method. This method supposes that calcium retention must be the same as that of a foetus of the same gestational age and it calculates the supply of calcium taking into account the assumed losses through skin, urine and stools. Based on this, they have calculated that premature neonates of 1000g and 1500g should receive 210mg and 185mg calcium per kilogram body weight per day, respectively. These in utero accretion rates are difficult

to achieve using conventional infant feeding methods of human milk or milk formulae. The premature infant on maximum oral feeds of 200ml/kg/day of human milk will ingest only 50-60mg/kg/day calcium (compared with approximately 200mg/kg/day required to match in utero accretion rates). Milk formulae specifically designed for use in premature neonates have increased calcium (and phosphorus) content in order to approximate in utero accretion rates. Table 6 summarizes the calcium and phosphorus contents of breast-milk and the commonly used milk formulae. Thus, the amount of calcium ingested by the neonate is less than that required to equal in utero accretion rates.

**TABLE 6.** Comparison of intrauterine calcium (Ca) and phosphorus (P) accretion rates, daily requirements with that supplied in human milk and various milk formulae at 200ml/kg/day. .

	Ca (mg/kg/day)	P (mg/kg/day)
In utero accretion rates	100-120	60-75
Recommended daily intake	200	110-125
Mature human milk	50-60	28-40
Humanized milk formula eg. S26 (Nestle)	80	40
Premature infant formula eg. Alpren	120	60
Soy-based formula	140	90

1.3.2.2. Absorption. The suboptimal amount of calcium ingested is further aggravated by the fact that calcium absorption in premature infants is rarely complete, ranging from 29-80% and is influenced by a multitude of factors. As mentioned previously, calcium absorption from the gut is both

active (1,25-dihydroxyvitamin D dependent) and passive. It appears that passive absorption does not play an important role in premature infants although it might do so in newborn rats. This evidence was suggested by Senterre et al (11) who demonstrated that in the absence of administered vitamin D, the amount of calcium absorbed by the premature infant is very small, even if calcium intake is increased. In contrast, the administration of vitamin D results in a clear augmentation of calcium absorption (11). The vitamin D supply of the premature infant must be adequate to maximize calcium absorption. The metabolism and requirements of vitamin D in the premature will be discussed in section 1.3.4.

Numerous other factors that affect calcium absorption must also be considered. Calcium absorption is increased by a high calcium intake (12), lactose containing formulae (mechanism unknown) (13) and increased gestational age. The amount and type of fat present in the intestinal lumen also affects calcium absorption. Increased steatorrhoea in the premature infant secondary to reduced bile salt concentration is accompanied by an increased faecal calcium loss (14). The reverse also holds true and an increased calcium supply aggravates fat malabsorption (15). This can be explained by the reduction in bile acid concentration in the neonate which decreases long chain saturated fatty acid absorption and these together with calcium form insoluble soaps. Chapell et al (15) showed that the replacement of long chain saturated

fats with medium chain or polyunsaturated fatty acids improved calcium absorption.

Calcium absorption is also affected by the amount of phosphate ingested relative to amount of calcium present. A calcium:phosphate ratio of 2:1 (as is found in human milk) appears to be optimum for calcium absorption, although milks with lower calcium:phosphorus ratio have similar percentages of calcium absorption.

### 1.3.3. Phosphorus in the premature neonate

1.3.3.1. Requirements. The amount of phosphorus in the foetus from 28-40 weeks rises from 3,4g to 16,8g corresponding to a daily accretion rate of 65-70mg/kg (Table 5) (8). Assuming that phosphorus retention in the neonate must be the same as that of the foetus in order to achieve in utero accretion rates, the estimated oral requirement of phosphorus in low-birth-weight babies is 110-125mg/kg/day (8, 16). Phosphorus content of human milk varies from 110-170mg/l (Table 6), thus at maximum feeds of 200ml/kg/day, 28-40mg/kg/day phosphate are ingested, which are insufficient to meet the in utero accretion rates to ensure both cell growth and incorporation of calcium and phosphate into bone.

1.3.3.2. Absorption. Phosphorus absorption in the premature and term neonate fed human milk or modified formulas is higher than that of calcium, ranging from 80-94% (11). It

seems to be independent of both vitamin D and calcium (11) as opposed to calcium absorption which is dependent on both vitamin D and phosphate supply.

#### 1.3.4. Vitamin D status and metabolism in premature infants

1.3.4.1. In utero. The foetus is dependent on maternal vitamin D status as placental transfer of maternal vitamin D and its metabolites occurs. There is a direct correlation between maternal and foetal 25-OHD at birth and it appears that this is the major vitamin D metabolite to be transferred across the placenta (17, 18). The source of foetal 1,25-(OH)<sub>2</sub>D is uncertain. Placental transfer has been demonstrated, but the majority of studies fail to show a correlation between maternal and foetal levels (18). The placenta and foetal kidney are capable of metabolizing transferred maternal 25-OHD and it appears that the foeto-placental unit supplies its own primary source of 1,25-(OH)<sub>2</sub>D (19). The role of these metabolites in placental calcium transport and foetal mineral homeostasis is unclear. After birth, there is a rapid rise in 1,25-(OH)<sub>2</sub>D under the influence of PTH (20). This increase is directly proportional to the concentration of 25-OHD and therefore reflects maternal vitamin D status. Low maternal and hence, low neonatal serum 25-OHD levels have been recorded in hypocalcaemic neonates (21), but the effect of maternal vitamin D status on foetal bone mineralization is less clear (22) as congenital rickets has been described.



1.3.4.2. Post-delivery. After delivery, the neonate has obviously lost the materno-placental unit as a source of vitamin D and is now dependent on dietary vitamin D as ultraviolet light exposure is minimal. The vitamin D requirements of the preterm newborn are controversial, but appear to be greater than those of the term neonate. Hoff et al (23) and Cifuentes et al (24) have reported rickets in premature babies receiving up to 400IU/day vitamin D which have healed on increased vitamin D supplementation. In a study by Hillman et al (25) a significant number of low-birth-weight infants showed biochemical and radiological evidence of rickets despite supplementation with 400IU/day vitamin D. Hillman et al (26) subsequently demonstrated that vitamin D requirements are better covered by a daily dose of 800IU/day. These infants had higher 25-hydroxyvitamin D, less hypophosphataemia and less radiographic evidence of osteopenia than those infants given 400IU/day.

It is uncertain why the premature infant has increased vitamin D requirements. Vitamin D status of the neonate at birth, reflecting maternal vitamin D status may partially explain the discrepant vitamin D requirements as found in the various studies (27). Immaturity in the pathway of normal vitamin D metabolism, i.e. either at intestinal, hepatic or renal level, may also account for increased vitamin D requirements and must be considered.

1.3.4.3. Absorption. Intestinal absorption of vitamin D seems adequate in the preterm infant (28), although this may not necessarily be so in the VLBW infant. Factors increasing vitamin D absorption are the dose of the vitamin administered, the presence of an acidic intraluminal environment and micelle formation which is essential for fat absorption. The premature infant has a lower intraluminal secretion of bile acids (29) (which are required for micelle formation), and a reduced efficiency of fat absorption, which may result in poorer vitamin D absorption.

1.3.4.4. Hepatic metabolism. Hepatic hydroxylation of vitamin D has been shown to be adequate in healthy preterm infants in a number of studies (30, 31). Glorieux et al (30), Robinson et al (31) and Salle et al (28) were able to demonstrate a rise in serum 25-hydroxyvitamin D levels in premature infants born after 28 weeks gestational age after administration of vitamin D (dosages ranging from 500-2100IU/day). Work by Hillman and Haddad (32), however, failed to show an increase in 25-OHD following vitamin D administration until 36-38 weeks gestational age, suggestive of immaturity of hepatic hydroxylation until that age. Hillman et al (26) later confirmed that serum 25-OHD levels did rise in premature infants given 400-800IU/day, but that these serum levels were lower than expected. Thus, decreased hepatic hydroxylation of vitamin D in premature infants does occur, but is of doubtful significance.

1.3.4.5. Renal metabolism. Renal hydroxylation of 25-OHD to 1-25(OH)<sub>2</sub>D in preterms also appears to be adequate. Plasma 1,25-(OH)<sub>2</sub>D has been demonstrated to increase significantly in premature infants (28-36 weeks gestational age) (33). Glorieux et al (30) confirmed a three-fold increase in 1,25-dihydroxyvitamin D levels in premature infants (32-37 weeks gestational age) after administration of high dose vitamin D. Serum 1,25-dihydroxyvitamin D levels have been found to be normal or even increased in preterm infants with rickets (34, 35), and Markestad et al (36) speculate that this represents a normal compensatory effort to ensure maximal absorption from a mineral poor diet.

1.3.4.6. Conclusion. Numerous studies on vitamin D metabolism in premature infants indicate minimal disturbance in function. Studies on vitamin D supplementation in this group of infants indicate that 800IU/day vitamin D should be the current recommended dose, as rickets has been described in VLBW infants receiving less than this (400IU/day vitamin D) and no advantages have been demonstrated in giving more than this amount.

#### 1.4. RICKETS

The VLBW infant not only has perturbations of mineral homeostasis, but frequently manifests features of metabolic bone disease including rickets.

#### 1.4.1. Definitions

- Rickets is a disorder of growing bones manifesting as a delay in mineralization of the cartilaginous matrix at the epiphyseal growth plate and of preformed osteoid at the metaphyseal regions of long bones.
- Osteomalacia refers to the failure of mineralization occurring in bone remodelled at the trabecular bone surface and within cortical bone.
- Osteopenia implies a generalized decrease in mineral content of bone.

The changes in bone tissue that occur concomitantly with vitamin D deficiency, or calcium and phosphate deficiency are failure of normal mineralisation of bone matrix, ultimately leading to an increased accumulation of unmineralized osteoid. These changes may be seen radiologically at the epiphyseal ends of long bones (particularly the wrist) as widening of the epiphyseal growth plate with cupping and fraying of the metaphyses, and in the diaphysis as thinning of the cortices and periosteal new bone formation.

#### 1.4.2. Aetiology

The aetiology of rickets may be broadly divided into two groups depending on whether the basic defect is a failure to maintain ionized serum calcium or phosphorus concentrations within the normal range for age.

The following table lists the causes of rickets which is applicable to all age groups. (Table 7).

**TABLE 7. Aetiological classification of rickets.**

Calcium deficient

**Inadequate formation of 1,25-(OH)<sub>2</sub>D**

**Vitamin D deficiency**

- dietary lack
- lack of sunlight exposure
- malabsorption syndromes

**Failure of hepatic hydroxylation**

- severe liver disease

**Increased vitamin D metabolism**

- anticonvulsant therapy

**Failure of renal hydroxylation**

- renal failure
- vitamin D dependency type 1

**Inadequate calcium intake**

**Dietary calcium deficiency**

**High fluoride intake**

Phosphate deficient

**Inadequate phosphate absorption**

**Dietary lack**

**Binding of dietary phosphate within the bowel**

**Increased renal losses**

**Renal phosphate leak**

- hypophosphataemic vitamin D-resistant rickets
- hypercalciuric vitamin D-resistant rickets
- tumour associated
- associated with neurofibromatosis
- associated with polyostotic fibrous dysplasia

**Complex renal tubular abnormalities**

- proximal tubular defect
  - primary: Fanconi's syndrome, Lowe's syndrome
  - secondary: galactosemia, Wilson's disease
- distal tubular defect
  - distal renal tubular acidosis

Miscellaneous

**Hypophosphatasia**

### 1.5. METABOLIC BONE DISEASE IN PREMATURE INFANTS

The improved survival of very low-birth-weight premature infants during the 1970's and 1980's has been accompanied by an increased incidence of metabolic bone disease (MBD) in this population. Also known as rickets of prematurity, this syndrome encompasses a spectrum of abnormalities of bone metabolism. This spectrum ranges from the severest form in which marked rickets and fractures occur, to the milder forms in which radiological rarefaction (osteopenia) and elevated alkaline phosphatase levels are found.

#### 1.5.1. Incidence

The exact incidence of metabolic bone disease is unknown because of the difficulty of diagnosis and the large number of subclinical cases. McIntosh et al (37) have shown that it becomes progressively more common and more severe with decreasing gestational age and birth weight and is particularly common in neonates of less than 1000g birth weight. Several studies have estimated a 30% incidence of MBD in preterm infants weighing less than 1500g at birth (38, 39). In neonates with a birth-weight of less than 1000g, reduced bone mineralization was demonstrated in about 75% of cases at 3 weeks of age (37, 39) and frank radiological rickets in 57% of these neonates (37). Rickets, fractures and bone demineralization in premature neonates usually present

between one and five months of postnatal age (40), with most cases presenting between two to four months postnatally (39). Several factors appear to be important in predisposing the premature neonate to the development of MBD (41):

1. It becomes progressively more common with decreasing gestational age (25,37).
2. It is most frequent in the breast-fed premature infants (42), but has been reported in infants on standard infant milk formulas (43) or soy milk preparations (40).
3. It occurs more frequently in infants who have had prolonged period of parenteral nutrition (44) or prolonged illness (4, 45).

#### 1.5.2. Diagnosis

The classical clinical signs of vitamin D deficiency rickets are frequently not apparent in the premature infant as MBD is often subclinical (39, 46). It may be an incidental finding on routine chest radiography (40, 47) or suspected in an infant with rib fractures, often subsequent to physiotherapy (47). The diagnosis may then be confirmed on biochemical data, radiography or bone histology.

1.5.2.1. Biochemical testing. Serum calcium and phosphate levels vary markedly and although these levels may be useful indicators of the presence of rickets, they are not diagnostic (4). Serum calcium concentration is often normal, but may vary from low to elevated, the latter usually

occurring in the presence of low serum phosphorus levels (38). Serum phosphorus concentrations are frequently low, but may also be within normal limits. Serum alkaline phosphatase (ALP) activity is also not a reliable indicator of rickets of prematurity. In most instances the ALP level will be raised (44), but its significance as an indicator of bone disease in the neonate is controversial due to the presence of non-bone isoenzymes. ALP is also produced in the liver (48) and concentrations are raised in the presence of liver disease, especially in obstructive jaundice in association with a raised gammaglutamyl transferase. In such situations ALP cannot be used as an indicator of bone disease unless isoenzymes are measured. In bone itself, ALP is an indicator of osteoblastic activity (48). The high serum ALP levels in preterm infants with or without rickets may thus reflect a normal response to a physiological increase in osteoblastic activity and bone mineralization associated with rapid growth. It has been suggested that ALP values of five times normal adult values may be normal in preterm infants. In a study by Glass et al (44), all patients with metaphyseal changes had maximum ALP activity greater than 750IU/l. Periosteal reactions were seen only if values exceeded 1000IU/l. Kovar et al (49) suggest that ALP values six times normal adult values indicate the need for radiological assessment and a value 7.5 times adult values is indicative of active rickets. The diagnosis of MBD based on abnormal ALP levels, must therefore be confirmed radiologically.



1.5.2.2. Radiological assessment. The radiological changes found in MBD are osteopenia, periosteal reactions and frank rickets. Various grading systems have been proposed in an attempt to standardize the radiological findings. Koo et al (39) have suggested the following classification which proves satisfactory in practice :

Grade 0 - Normal bones.

Grade 1 - Rarefaction only, i.e. loss of dense white line at metaphyses, increased submetaphyseal lucency and cortical thinning.

Grade 2 - Bone end changes (fraying and cupping of metaphyses), subperiosteal new bone formation.

Grade 3 - The above changes with fractures.

1.5.2.3. Photon absorptiometry. This is probably the best technique currently available for measuring bone mineral content, but is largely a research tool as equipment is expensive and not generally available. A number of studies have shown this method to be a reliable measurement of BMC in preterm infants as well as a means of monitoring changes of BMC with increasing age and different feeding regimes (50, 51). However, as single photon absorptiometry only measures the mineral content of bone, it does not distinguish between osteoporosis and rickets.

### 1.5.3. Differential diagnosis

After a careful clinical examination, biochemical and radiological investigations, there are few other conditions that resemble rickets of prematurity. Trace mineral deficiencies may occur in neonates with complicated postnatal courses in particular with prolonged hyperalimentation. Of these, copper deficiency may present with similar radiological changes to those found in rickets of prematurity. The changes of copper deficiency include osteopenia, subperiosteal new bone formation and blurring and cupping of the long bone metaphyses (52, 53). Biochemical investigations will differentiate between these two conditions as in copper deficiency, serum calcium and phosphate concentrations are normal, but serum copper and caeruloplasmin concentrations are decreased.

### 1.5.4. Pathogenesis

Several pathogenetic mechanisms have been suggested to explain the prevalence of bone disease in premature infants and can be grouped as follows :

- a. Vitamin D deficiency or abnormalities of vitamin D metabolism.
- b. Dietary calcium and/or phosphorus deficiency.

Some of the factors that may be involved in the pathogenesis of MBD are listed in Table 8 and discussed

below. These factors are, in turn, influenced by vitamin D supplementation, type of milk feed, severity of postnatal problems, prolonged use of parenteral hyperalimentation and furosamide therapy as well as other as yet unidentified factors.

**TABLE 8.** Aetiological factors in metabolic bone disease of prematurity.

- |  |
|--|
| <p><b>Calcium and phosphorus deficiency</b></p> <ul style="list-style-type: none"> <li>- human milk, standard formula and soy feeds</li> <li>- prolonged hyperalimentation</li> <li>- fat malabsorption</li> <li>- diuretics</li> <li>- systemic disease and acidosis</li> </ul> <p><b>Vitamin D related disorders</b></p> <ul style="list-style-type: none"> <li>- dietary intake deficiency</li> <li>- hepatic hydroxylation of vitamin D</li> <li>- renal hydroxylation of vitamin D</li> <li>- end organ responsiveness</li> </ul> |
|--|

#### 1.5.4.1. Role of vitamin D

A detailed description of vitamin D metabolism in the premature baby has been discussed (section 1.3.4). No major abnormalities in the absorption, hepatic or renal hydroxylation of this vitamin in premature infants have been delineated. Vitamin D intakes of 800-1000IU/day may be required to ensure adequate vitamin D absorption, hepatic hydroxylation and optimum calcium absorption. Despite adequate supplementation, biochemical evidence of rickets has been documented in VLBW infants.

Thus there is little evidence that vitamin D is the primary problem in the aetiology of metabolic bone disease in this

group of infants, although a deficiency of vitamin D, if adequate intake is not maintained, might increase the prevalence.

#### 1.5.4.2. Role of calcium and phosphorus

Evidence of rickets in vitamin D replete premature infants as well as minimal dysfunction in vitamin D metabolism is suggestive of substrate (calcium and/or phosphorus) deficiency (56). As has been previously discussed, many premature infants of 25-36 weeks gestation can grow at a rate similar to the in utero growth rate and have similar calcium (100/120mg/kg/day) and phosphorus (60-75mg/kg/day) requirements as the foetus in order to maintain adequate bone mineralization. Human milk, at an intake of 150-200ml/kg provides only 51-68mg/kg/day calcium and 21-28mg/kg/day phosphorus (Table 6). Numerous studies have documented the development of clinical and biochemical rickets in preterm and very low-birth-weight infants fed breast milk (54, 55, 57, 58), suggesting that human milk alone is inadequate to meet the mineral needs of the growing preterm infant.

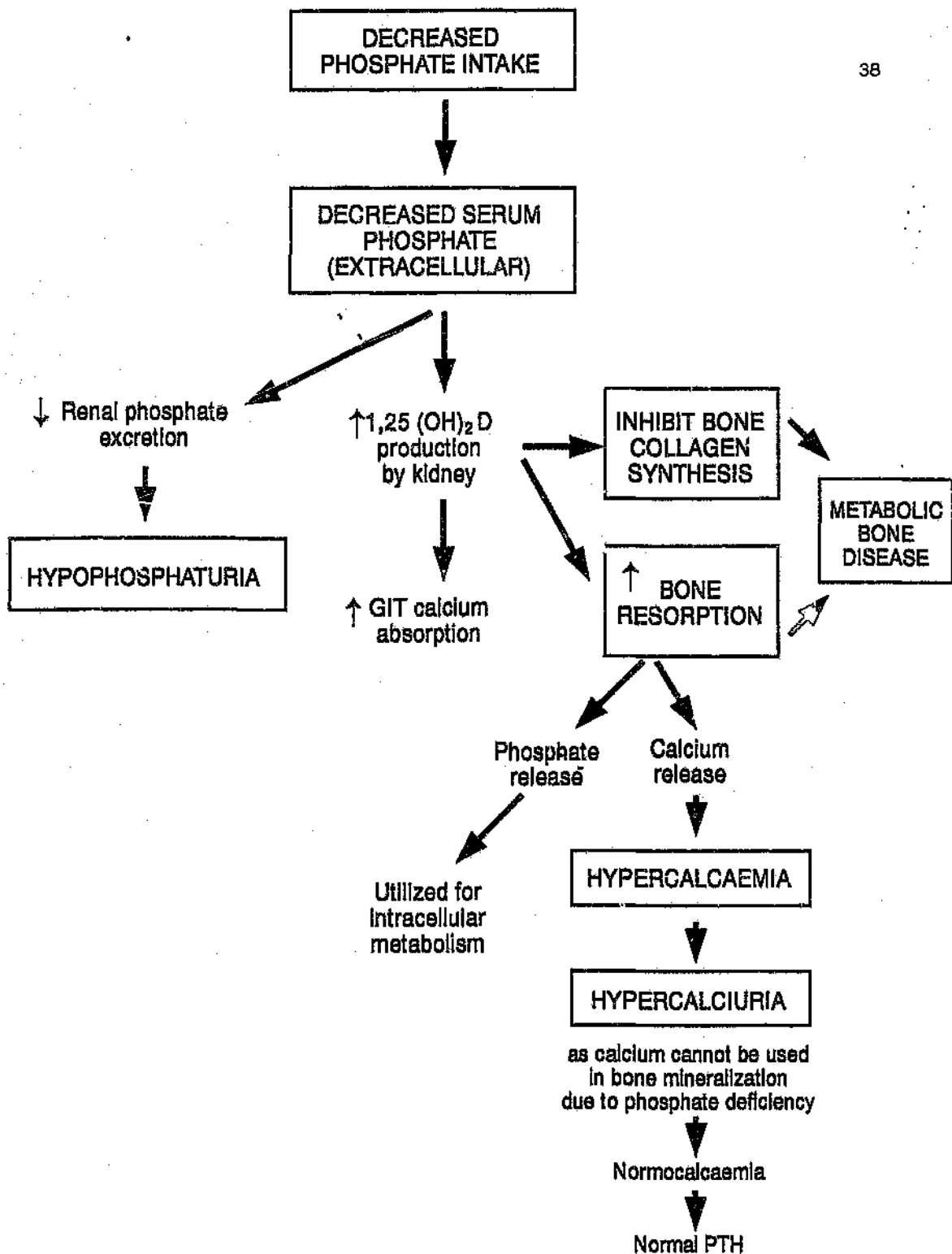
VLBW infants, especially those weighing less than 1000g (25), have been shown to develop metabolic bone disease when fed standard milk formulae. The calcium and phosphorus content of these formulae are also insufficient to meet the bone mineralization requirements of the VLBW infant (Table 6). Several reports have documented a decrease in MBD in VLBW

infants fed with calcium and phosphorus supplemented feeds (43, 59, 60) suggesting that both calcium and phosphorus deficiency may play a role in the pathogenesis of MRD. Recent studies suggest that phosphorus depletion may play a more significant role in this disease entity (57, 58, 61-63). Rowe et al (62) showed that in preterm infants fed human milk compared with those fed standard formula the serum phosphate is significantly lower, the renal excretion of phosphate is virtually zero (compared with 40% in the controls) and the renal excretion of calcium is higher despite having received one-half to two-thirds the amount of calcium. Supplementation of the human milk fed infants with oral phosphate (0.8mmol/kg/24hr) reversed these findings resulting in reduced urinary calcium loss, increased renal phosphate excretion, and elevated serum phosphate without decreasing serum calcium concentrations. PTH values remained normal (64). These studies support the hypothesis of phosphate deficiency syndrome in VLBW infants which is characterised by the biochemical findings of hypophosphataemia, hypophosphaturia and hypercalciuria (63) (Table 9).

**TABLE 9.** Biochemical findings in phosphorus deficiency.

Hypophosphataemia
Hypophosphaturia / High TmP/GFR
Hypercalciuria
Increased serum alkaline phosphatase
Normal (or increased) serum calcium
Normal serum PTH

The pathophysiologic consequences of phosphate deficiency on bone mineralization are due to both direct and indirect effects of hypophosphataemia (63). Low extracellular phosphate levels may directly enhance bone resorption as well as decrease matrix formation and bone mineralization (63). The indirect effects of hypophosphataemia are depicted in Figure 6. Decreased phosphorus ingestion leads to a decrease in serum phosphorus concentration and virtually complete renal tubular phosphate reabsorption. The low extracellular phosphate concentration increases renal 1-alpha-hydroxylase activity resulting in elevated serum  $1,25-(OH)_2D$  levels. This stimulates bone resorption (releasing calcium and phosphate) and increases intestinal calcium absorption. In the VLBW infant this increased calcium may not be utilized in bone mineralization as phosphate is deficient and the "excess" is excreted in the urine. Serum phosphate remains low despite its release from bone as it is an essential intracellular functions (65). Elevated  $1,25-(OH)_2D$  inhibit in vitro bone collagen synthesis which may contribute to MBD. Serum PTH levels remain within the normal range as does serum calcium (which is the main stimulus for its release). The effects of phosphorus and  $1,25-(OH)_2D$  on bone are thus independent of PTH.



**Figure 6 :** The pathophysiologic effects of hypophosphataemia

The demonstration of the development of hypophosphataemic rickets manifesting with the predictable biochemical pattern (Table 9) in numerous studies on premature infants fed human milk (54, 57, 58) and standard milk formulas (25), suggests that in the VLBW infant fed human milk or standard milk formulas there is insufficient phosphorus available for normal bone mineralization and matrix formation.

The use of soy-based formulas appears to aggravate this problem despite their slightly higher calcium and phosphorus content (Table 6). The probable reason for this is the presence of phytates in these milks which form unabsorbable complexes with phosphorus, decreasing its bioavailability and intestinal absorption (66).

In view of the fact that hypophosphataemia seems to be the most prominent feature of MBD, supplementation with phosphorus alone has been attempted (57). However, hypocalcaemia was observed (57) despite adequate vitamin D intake. Greer et al (59) have recommended calcium and phosphorus supplementation as the VLBW infant is calcium as well as phosphorus deficient.

Factors that may compound the risk of mineral deficiency in preterm infants must also be considered:



- a. Systemic disease and acidosis. Callenbach et al (4) and Bosley et al (45) found a greater prevalence of MBD in the sicker preterm infant. Acidosis secondary to sepsis, poor perfusion and systemic disease may further contribute to MBD by increasing bone resorption.
- b. Total parenteral nutrition has been well documented as a cause of rickets in VLBW infants requiring parenteral alimentation for prolonged periods (67). Inadequate supply of calcium and phosphorus in the solutions administered and difficulty in increasing the concentrations of these ions plays a primary aetiological role (68).
- c. VLBW infants requiring ventilatory support for hyaline membrane disease have a higher prevalence of rickets, often presenting with incidental rib fractures on chest radiographs. Decreased mineral intake as well as relative fluid restriction, probably play a major role.
- d. MBD has been documented in premature infants receiving prolonged furosemide therapy (69). Furosemide, a potent loop diuretic which increases urinary calcium excretion, is an aggravating factor compounding the effects of poor nutritional intake, acidosis and hyperalimentation.

#### 1.5.5. Calcium and phosphorus supplementation

It is thus evident from the above discussion that external sources of mineral supply in either human milk, standard formulae or soy formulae are inadequate in the fast growing VLBW infant whose needs approximate those of the foetus.

Provision of additional calcium and phosphorus, i.e 210mg/kg and 90mg/kg daily intake of calcium and phosphorus respectively to the VLBW infant to meet the in utero accretion rates, is a challenge.

Numerous studies have been performed on the supplementation of calcium and phosphorus in premature infant feeding. Hypocalcaemia has been observed following phosphorus supplementation (57, 58) and Greer et al (70) have suggested that both phosphorus and calcium supplements are necessary. Lindroth et al (71) showed a decrease in radiological rickets in breast-fed infants below 1000g if calcium and phosphorus were supplemented.

Practical problems occur when calcium and phosphorus are supplemented and care must be taken in choosing the mineral preparation. Calcium gluconate or gluceptate in liquid form and a mixture of mono and dibasic phosphate salts seem to be the most appropriate (72). Simultaneous supplementation of both minerals may result in the formation of insoluble salts, while alternate administration of the minerals is likely to be complicated by high urinary loss of the supplemented mineral. These problems may be avoided by adding phosphate to the milk first and mixing thoroughly prior to the addition of calcium (72).

These problems have been addressed in the development of

specialized premature formulas and breast-milk fortifiers. Different authorities have recommended that phosphorus content should not be less than 20, 25 or 30mg/100kcal in formulae adapted for premature neonates. Two or three times these concentrations may be given provided the calcium:phosphorus ratio remains between the limits of 1.1: and 2.2:1.

It may also be argued, however, that most premature infants do not require special supplementation for the following reasons (41) :

1. Although biochemical evidence of impaired mineralization is a common finding in breast-fed premature infants, most do not develop any clinical complications related to these changes.
2. Furthermore, biochemical abnormalities return to normal by 3-4 months of age.
3. Follow-up of these infants does not demonstrate any long term sequelae.

These findings are probably true, but it is the sick VLBW infants weighing less than 1000g at birth who are at greatest risk of developing MBD and its complications, and attention should be given to preventive therapy in this group.

#### 1.5.6. Recommendations

1. All VLBW infants should receive 800IU vitamin D daily from two weeks post-delivery.
2. Specialized premature formulas should be used in VLBW infants on formula feeds.
3. Those infants who are breast-fed should be carefully monitored for the development of MBD and the appropriate minerals supplemented if MBD develops.
4. Soy milk formulas should not be used in VLBW infants except in exceptional circumstances.
5. VLBW infants receiving prolonged total parenteral nutrition and with severe postnatal complications should be monitored for MBD and treated accordingly.

#### 1.5.7. SUMMARY

In light of these conflicting views, this study was undertaken to assess the extent of the problem of metabolic bone disease in VLBW infants born at Baragwanath Hospital, and also to determine the most appropriate feeds for these infants and the need for active intervention.

### THE BARAGWANATH PERSPECTIVE

The maternity section of Baragwanath Hospital is responsible for a total of 36000 deliveries annually - 24000 in-hospital and 12000 clinic deliveries.

Low-birth-weight infants (<2500g) constitute 14% of all deliveries and very-low-birth-weight infants (<1500g) approximately 2%. Of the VLBW infants 40% are SGA and 60% are AGA. The Neonatal Intensive Care Unit at Baragwanath Hospital has 12 ventilator beds and the Transitional Care Unit has the facilities for the management of a further 25 neonates who are extremely ill, but do not require ventilation. In view of the large number of deliveries at this hospital and the relatively few intensive care beds available, it is the policy of the Neonatal Unit not to ventilate neonates weighing less than 1000g at birth, but to administer all other therapy as indicated. Neonates weighing more than 1000g are ventilated according to standard indications. Survival of neonates weighing less than 1000g is approximately 20%. The high mortality is thought to be multifactorial including the high incidence of unbooked mothers who are admitted in advanced labour, the high incidence of asphyxia and the policy of not ventilating this group of neonates.

Feeding policies for neonates at Baragwanath Hospital which serves the developing community of Soweto are aimed at promoting breast-feeding. Mothers of VLBW or LBW neonates

generally stay in hospital with their infants and are encouraged to express breast milk which is then used to feed these neonates. Breast feeding is introduced from 1800g and bottle feeds gradually weaned until the infants are fully breast fed prior to discharge from hospital at approximately 1900g.

## CHAPTER 2

### METHODS

#### 2.1. Infant Selection

All neonates weighing less than 1200g born at Baragwanath Hospital were entered into the study at birth. The cut-off weight of 1200g was chosen as this group of neonates is at greatest risk of developing MBD. Exclusion criteria were the presence of congenital abnormalities (chromosomal and skeletal), congenital infections or any other diseases which could themselves cause bone disease. Neonates dying within the first two weeks post-delivery were subsequently excluded as they were unlikely to have developed MBD at this age.

The study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand (protocol no. 8/3/87). Informed consent was obtained from the mothers of the neonates prior to inclusion in this study. Gestational age was assessed according to maternal dates and the Ballard score within 48 hours of delivery (73).

#### 2.2. Infant feeding

The infants were fed according to the routine feeding practices of the Baragwanath Hospital Neonatal Unit. Parenteral fluids administered initially consisted of a 10% dextrose water solution without additional potassium, but

containing 0.23mg/ml calcium. Potassium containing electrolyte solutions which also contained 0.1mg/ml calcium and 0.12mg/ml phosphorus were introduced after 48 hours unless contra-indicated. Parenteral feeds in the form of amino acid and dextrose mixtures were introduced from the fourth day of life if indicated by clinical condition. These feeds contained 0.43 kcal/l, 0.07 mg/ml calcium and 0.08 mg/ml phosphorus.

Enteral feeds consisted of own mother's milk until two weeks post-delivery when the surviving neonates were entered into the active phase of the study. At this time, the neonates were randomised into two groups according to hospital number. Those with even numbers received own mother's milk only (OMM or control group) and those with odd numbers received own mother's milk mixed in equal proportions with a premature formula of Alprem (mixed feed or MF group) making up the study group. Alprem contains 0.7 kcal/ml, 0.55 mg/ml calcium and 0.3 mg/ml phosphorus. The relevant constituents of the milk feeds are summarised in Table 10. Feeds were administered three hourly and increased gradually to a maximum of 180-200 ml/kg/day. Once the neonates reached 1800g, the mothers were encouraged to breast feed, and the provision of Alprem was stopped, thus a weight of 1800g, was used as the end point of the randomized study, although the infants were followed up for 3 months after discharge.



**TABLE 10.** Calcium, phosphorus and energy content of own mother's milk (OMM), Alprem and mixed feeds (MF).

CONSTITUENTS	OMM	Alprem	MF
Calcium (mg/l)	255	550	400
Phosphorus (mg/l)	170	300	240
Protein (g/l)	12	24	18
Energy (kcal/l)	650	700	680
Fat (g/l)	33	33	33

Vitamin D supplementation of 800 IU/day from Day 14 was administered, as well as oral iron supplementation from one month post-delivery.

### 2.3. Monitoring and management

Management of the neonates was determined by the clinician in charge of each patient. Postnatal complications (prolonged jaundice, necrotizing enterocolitis and duration of ventilation) were recorded. Volumes and types of parenteral and enteral fluids were recorded daily and from this daily calcium, phosphorus and energy intakes were calculated.

Biochemical assessment of bone status which included serum total and ionized calcium, phosphorus, magnesium, alkaline phosphatase and albumin were performed at two weekly intervals until the infants weighed 1800 g. Thereafter, the above parameters were performed at six weekly intervals for three visits following discharge. Growth parameters of weight, height and skull circumference were also determined at the time of these biochemical investigations. Serum 25-OHD

and  $1,25-(OH)_2D$  estimations were done at two weeks post-delivery, on discharge from hospital (1800 - 1900g) and at the third (final) follow-up visit. Measurement of total serum calcium and magnesium concentrations were performed by atomic absorption spectroscopy using the Varion Techtron Atomic Absorption Spectrometer and ionized calcium by specific calcium electrode using the Orion ionized calcium analyser SS-20. Serum inorganic phosphate, alkaline phosphatase and creatinine levels were assayed utilizing a Technicon Autoanalyser II according to methods described by Horst et al (74), Morgenstern et al (75) and Chasson et al (76) respectively. Serum albumin levels were assayed by the Bromocresol green method of Doumas et al (77), 25-OHD according to methods described by Haddad and Ckyu (78) and  $1,25(OH)_2D$  by microassay described by Reinhardt et al (79).

Radiographs of wrists and distal femur were obtained on discharge from hospital and at the final follow-up visit or if the alkaline phosphatase level was greater than 750 IU/l at any stage. Radiographs were reviewed blindly and the presence of periosteal reactions, osteopenia and/or rickets recorded.

Data was analysed using analysis of variants and Chi-square procedures.

### CHAPTER 3

#### RESULTS

##### 3.1. Clinical characteristics of control and study groups

A total of 121 neonates weighing less than 1200g at birth and born at Baragwanath Hospital between February and October 1987 were assessed as possible candidates for inclusion in the study. There were 56 survivors at two weeks postgestational age and these were then entered into the randomized trial. Those neonates who died before two weeks of age were not included in the study. Of the 65 neonates who died prior to two weeks, only 32 were assessed for gestational age prior to their deaths. The major reason for the lack of gestational age assessment of this group was the severity of illness and the short duration of survival. Fifteen of the 32 (47%) were appropriate for gestational age compared with 8/56 (14%) surviving neonates ( $p=0.002$ ). The majority of the deaths were due to extreme prematurity.

Of the survivors, 27 received own mother's milk (OMM) and 29 received mixed feeds (MF). Three infants in the OMM group were subsequently excluded as they had received incorrect feeds. Table 11 summarizes the characteristics of the neonates in each group. There were no statistically significant differences between the two groups in the parameters assessed at birth or at two weeks of age when they entered the randomised trial.

**TABLE 11.** Comparison of the clinical characteristics of the neonates in the control (OMM) group and study (MF) group.

	OMM GROUP	MF GROUP
No. in randomized study	29	27
No. completing in hospital study	29	24
No. completing follow-up	16	14
Mean gestational age (wks)	32.8 $\pm$ 1.3	32.6 $\pm$ 1.3
Mean birth weight (gm)	1095 $\pm$ 99	1091 $\pm$ 80
AGA	4	4
SGA	25	20
Multiple pregnancies	4	2
Ventilated (n)	3	2
Septicaemia (n)	6	3
Exchange transfusion (n)	3	2
Enterocolitis (n)	1	1
Hospital stay (days)	52 $\pm$ 11	55 $\pm$ 14

### 3.2. Anthropometric results

Weight gain was similar in control and study groups as shown in Figure 7. Both groups followed the expected pattern of weight gain when plotted on the Dancis Premature-Birth Weight Chart (80). Minimal weight loss occurred for the first ten days. This was then followed by weight gain which appeared to plateau after week 5 and increase again following discharge from hospital.

Growth, as assessed by increase in length and skull circumference was not statistically different in the control and study groups as shown in Figures 8 and 9. The changes in both these parameters followed a similar pattern to those found in weight, although the infants who had received the mixed feed while in hospital were statistically longer than the breast-fed group at the second and third follow-up

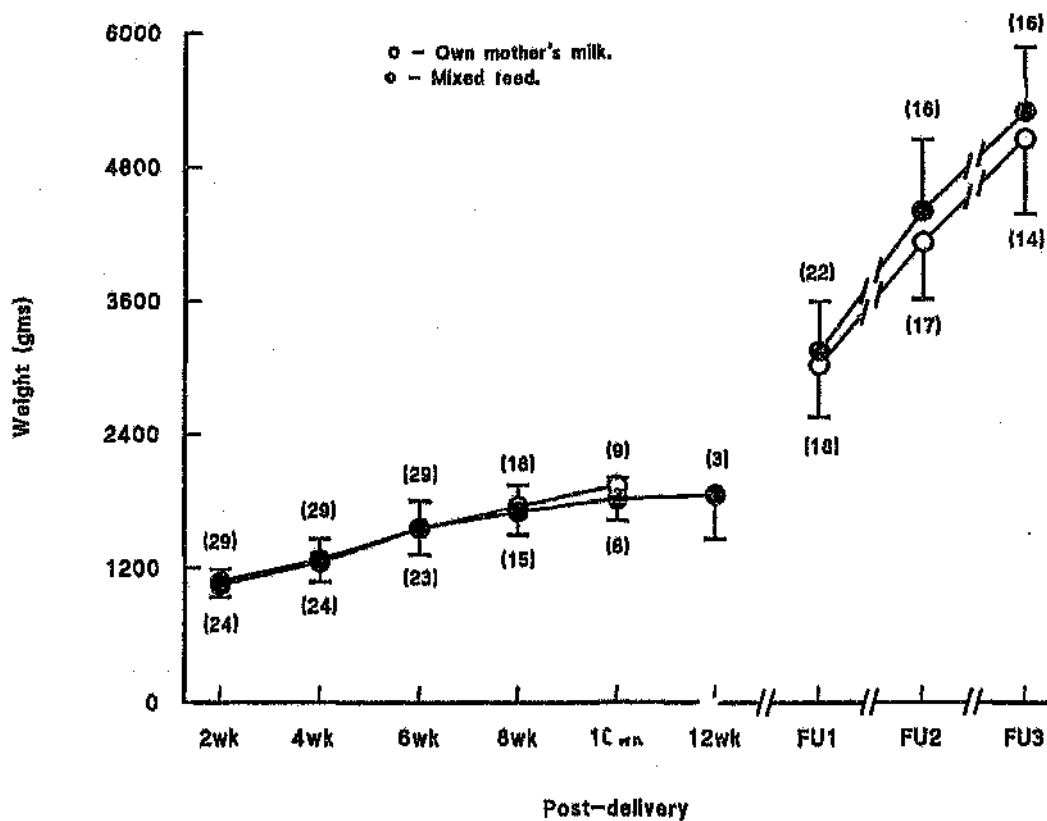


Figure 7. Changes in weight during study period in control and study groups.

(FU1 = 6 weeks post discharge; FU2 = 12 weeks post discharge;

FU3 = 18 weeks post discharge). (n) = no. in each group.

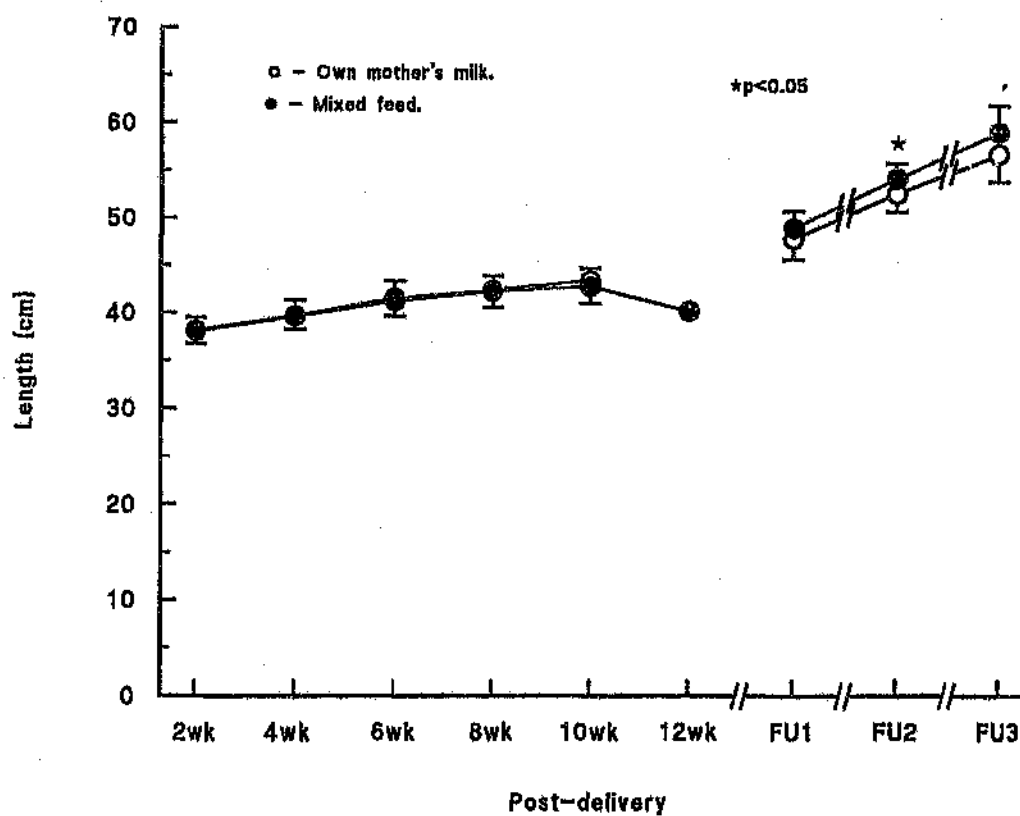


Figure 8. Changes in length during study period in control and study groups.  
(FU1 = 6 weeks post discharge; FU2 = 12 weeks post discharge;  
FU3 = 18 weeks post discharge).

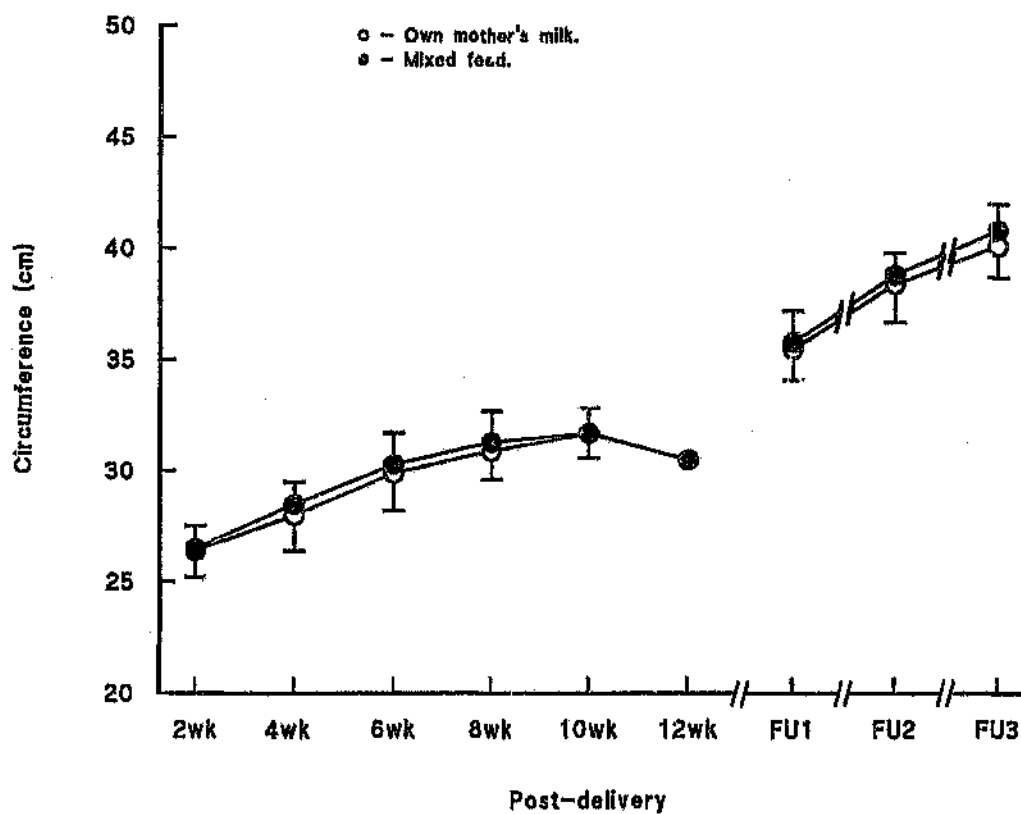


Figure 9. Changes in skull circumference during study period in control and study groups. (FU1 = 6 weeks post discharge; FU2 = 12 weeks post discharge; FU3 = 18 weeks post discharge).

### 3.3. Energy, calcium and phosphorus intakes

These intakes were calculated according to the various milk constituents as shown in Table 10.

#### 3.3.1. Energy intake (Figure 10).

The energy content of the milk feeds used in the control and study groups was only slightly different (650 and 680kcal/l respectively; Table 10), therefore a significant difference in energy intake between the two groups was not anticipated. Energy intake was minimal in the first week post-delivery and then increased progressively over the next two weeks as the volume of milk intake increased. Energy intake had reached a plateau by four weeks post-delivery at approximately 130kcal/kg/day once maximum milk intakes of 200ml/kg/day were administered.

#### 3.3.2. Calcium intake (Figure 11).

Calcium intakes in the control and study groups were minimal in the first week of life and increased sharply over the next two weeks as the volume of feeds increased. The gradient of increase was greater in the study group which reached a plateau at an intake of approximately 80mg/kg/day from 4 weeks of life. The control group reached a plateau at a similar time but at a lower intake of 50mg/kg/day. The differences in intakes between the two groups was statistically significant ( $p < 0.0001$ ) from week 3 until discharge from hospital.



### 3.3.3. Phosphorus intake (Figure 12).

Phosphorus intakes followed a similar pattern to that of calcium intakes. The phosphorus intake was significantly greater in the study group reaching a steady state at 45-50mg/kg/day compared with 35mg/kg/day phosphorus intake in the control group ( $p < 0.0001$ ).

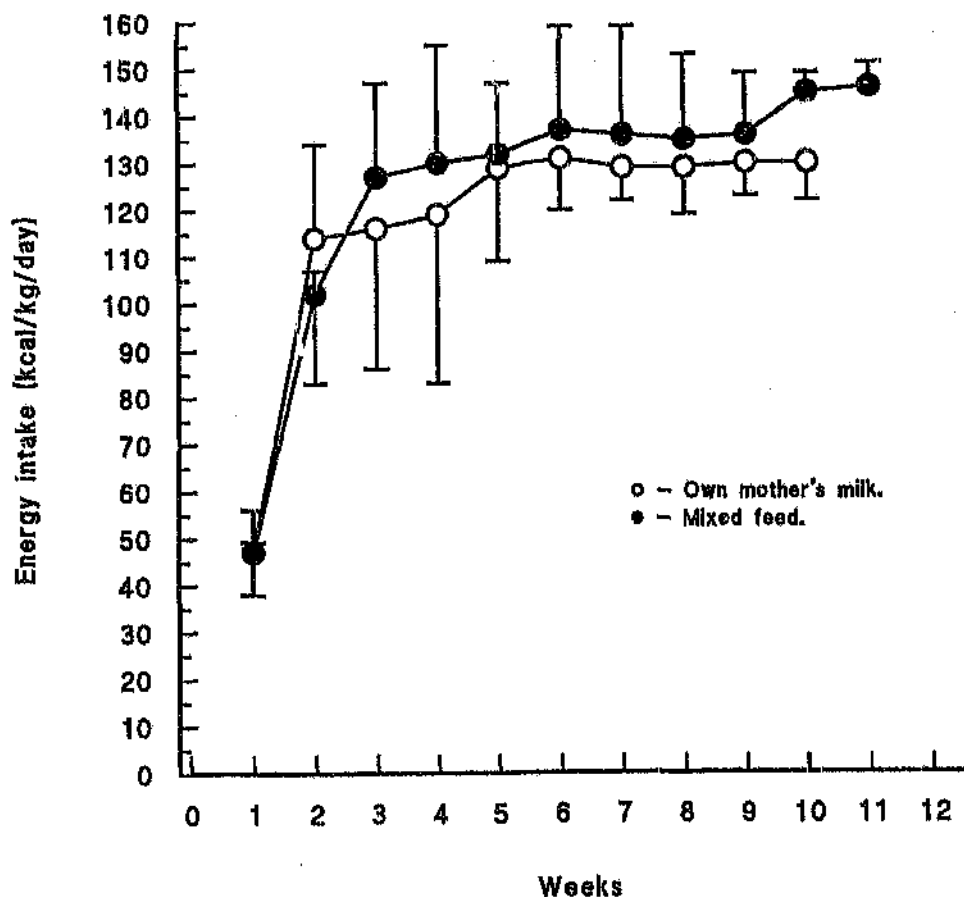


Figure 10. Energy intake during hospitalization in control and study groups.

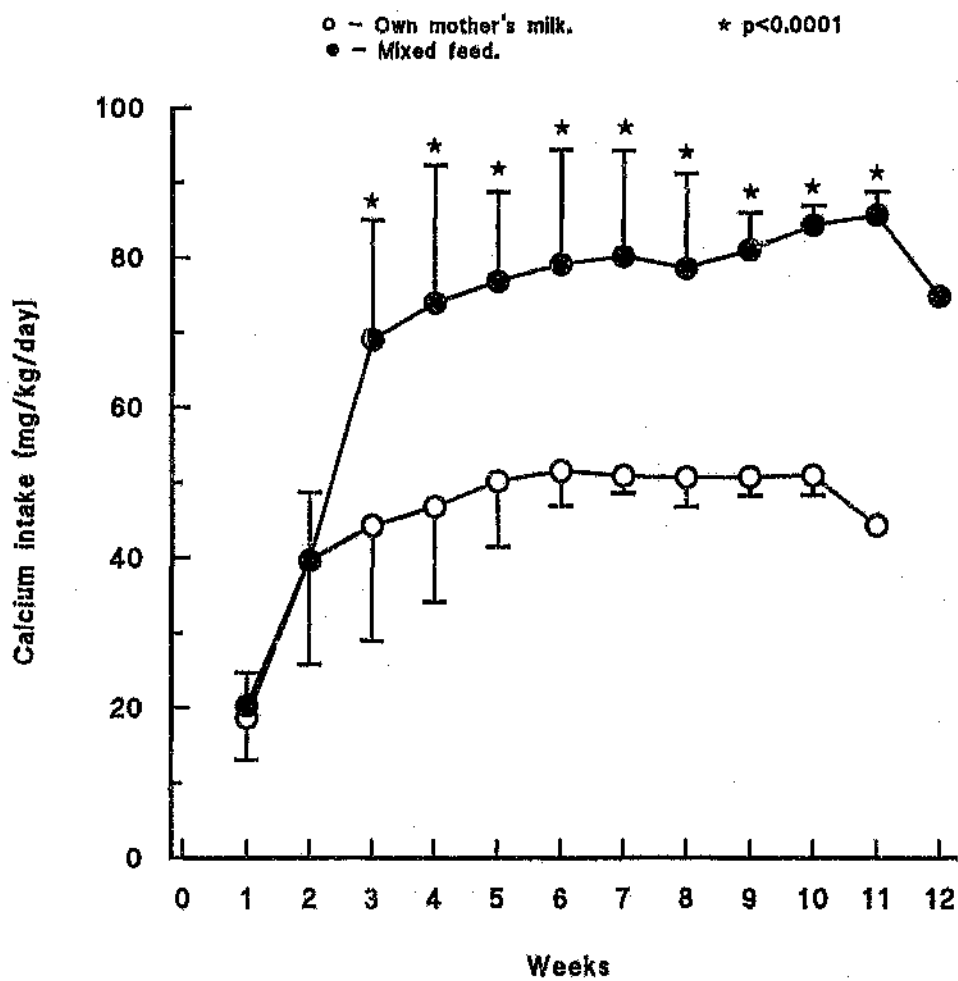


Figure 11. Calcium intake during hospitalization in control and study groups.

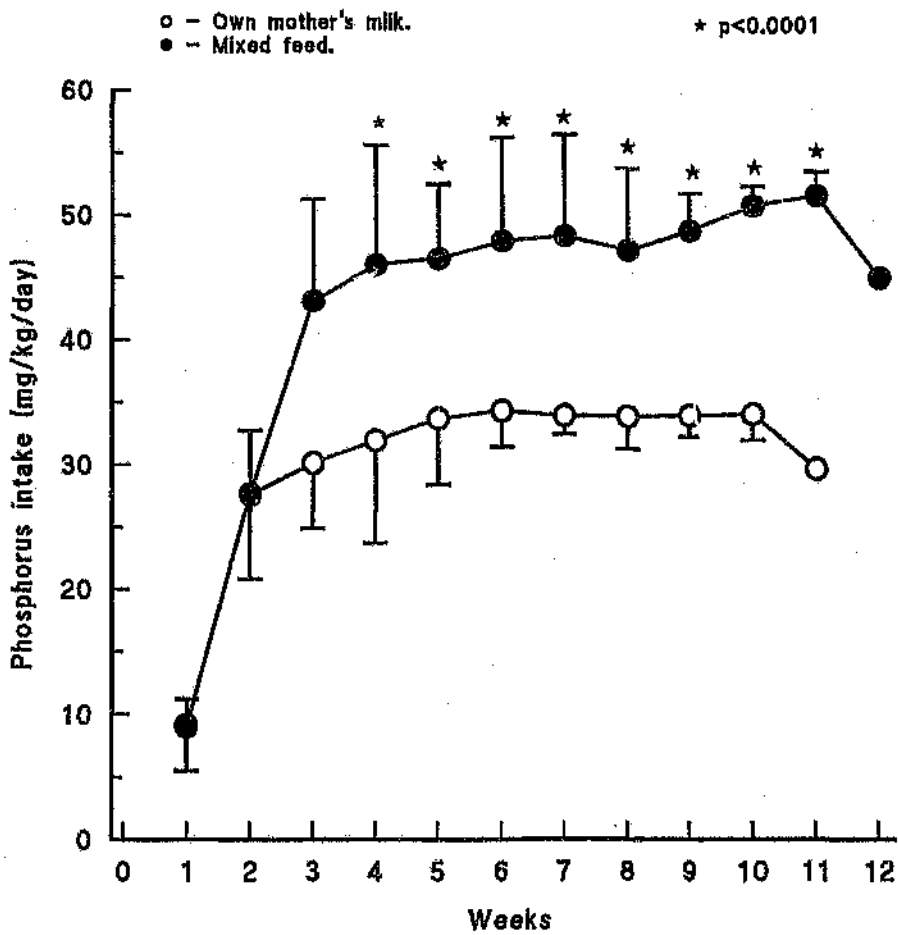


Figure 12. Phosphorus intake during hospitalization in control and study groups.

### 3.4. Biochemical Findings

#### 3.4.1. Serum albumin levels (Figure 13).

Serum albumin levels were initially similar in both groups. Values in the study group remained relatively constant throughout the hospital stay, while in the control group mean levels fell from six weeks of age. The study group had significantly higher values than the control group at weeks eight and ten. After discharge, values in both groups rose progressively to reach normal paediatric values.

#### 3.4.2. Serum magnesium levels (Figure 14).

Magnesium levels remained stable and within normal limits throughout the study period in both groups.

#### 3.4.3. Serum total and ionized calcium concentrations (Figures 15, 16).

There was no statistically significant difference in serum calcium (total and ionized) levels between the control and study groups, despite different calcium intakes (Table 10). The graphs of the mean serum concentrations of total and ionized calcium follow similar curves indicating that total serum calcium levels were not affected by changes in serum albumin. Serum ionized calcium levels in both OMM and MF groups were lowest at 2 weeks of age (approximately 2.05mEq/l) and increased gradually until 8 weeks of age (about 2.2mEq/l). The levels then remained stable for the remainder of the in-hospital stay. Following discharge, serum

ionized calcium levels increased further ( $2.4 \pm 0.1\text{mEq/l}$ ) to reach normal paediatric values.

#### 3.4.4. Serum phosphorus levels (Figure 17).

Serum phosphorus levels decreased post-delivery ( $2.1 \pm 0.4\text{mmol/l}$  at 2 weeks) to their lowest level at 10 weeks ( $1.58 \pm 0.3\text{mmol/l}$ ). Levels rose again following discharge from hospital, and remained constant during the follow-up period. There were no statistically significant differences between the two groups, despite higher phosphorus intakes in the study group.

#### 3.4.5. Serum alkaline phosphatase levels (Figure 18).

Serum alkaline phosphatase levels increased progressively in both the own mother's milk and mixed feed groups reaching peak values at 10 weeks post-gestational age. As the values at each time point were not normally distributed, the data were log transformed and these values compared between the two groups. The own mother's milk (control) group tended to have higher alkaline phosphatase levels during the in-hospital study period. This difference was highly significant at the time of discharge from hospital ( $t=4.3$ ;  $p<0.0001$ ). No difference was found at the time of entry or discharge from the study. Using a cut-off level of  $750\text{IU/l}$  AP as highly suggestive of rickets, the OMM group had a greater number of infants above this level, but this did not reach statistical significance (Chi-square 3.01;  $p=0.08$ ).

### 3.5.6. Serum 25-hydroxyvitamin D levels (Figure 19).

Serum 25-OHD values were measured at two weeks post-delivery, on discharge from hospital and again at discharge from the study. Values were within the normal range (10-50ng/ml) at all times. In the control group, values remained constant while the infants were in hospital, but then rose sharply ( $p < 0.0004$ ). In the study group, levels rose progressively at the three time points ( $p < 0.0001$  and  $0.007$  respectively). At entry into the study (two weeks of age), the control group had significantly higher values than the study group ( $p < 0.04$ ), while at hospital discharge, the pattern had reversed with the study group having higher values than the control group ( $p < 0.01$ ). At follow-up, the levels in the two groups were similar.

### 3.4.7. Serum 1,25-dihydroxyvitamin D levels (Figure 20).

Serum 1,25-(OH)<sub>2</sub>D, measured at the same times as 25-OHD followed a similar trend in both groups. There was an overall two-fold increase in 1,25-(OH)<sub>2</sub>D levels which occurred from the time of entry into the study to hospital discharge ( $p < 0.01$  in both groups). Values then tended to decrease.

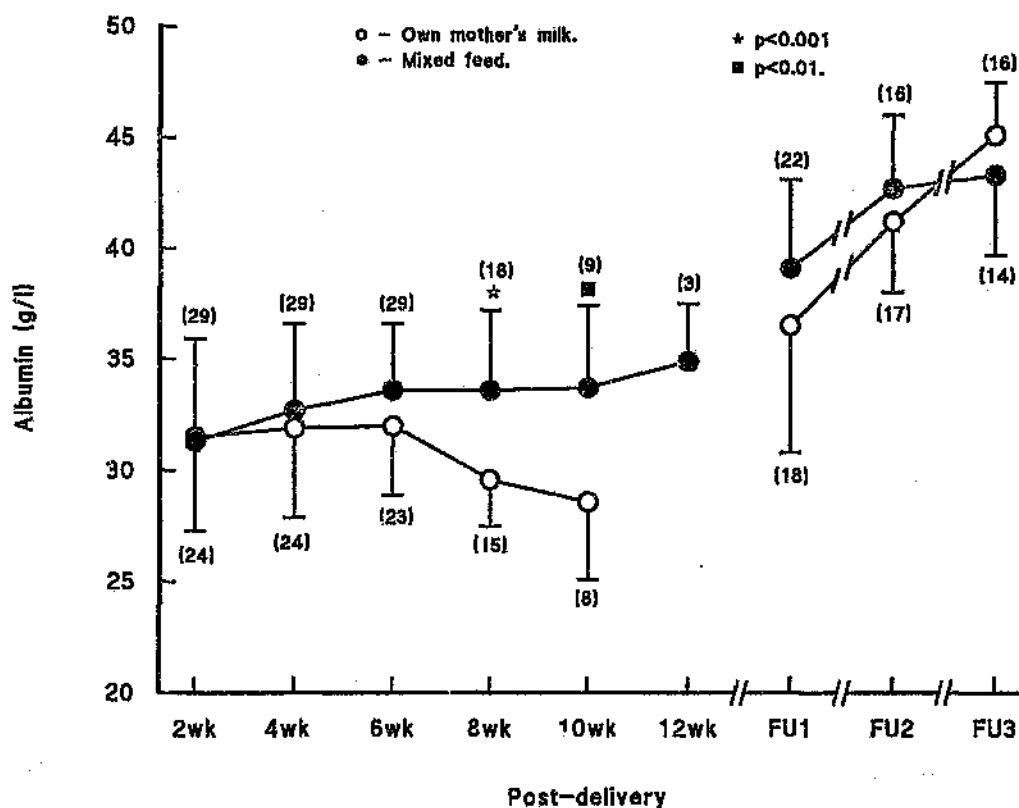


Figure 13. Serum albumin concentrations during study period in control and study groups.

(FU1 = 6 weeks post discharge; FU2 = 12 weeks post discharge;

FU3 = 18 weeks post discharge). (n) = no. in each group.

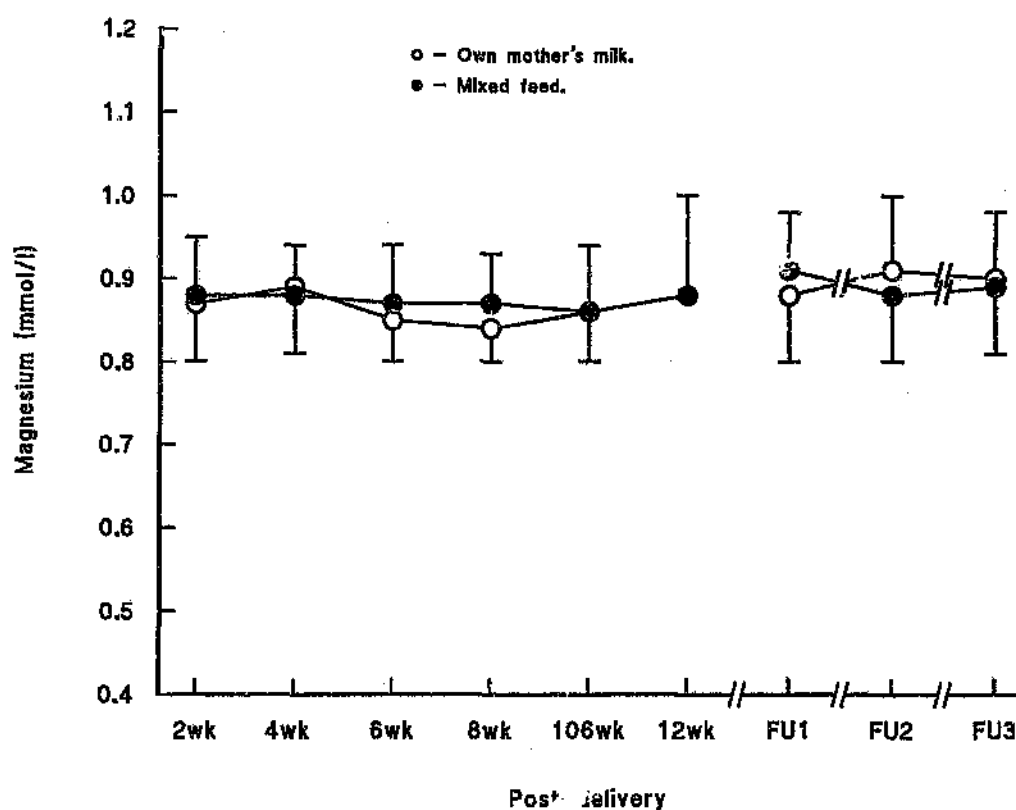


Figure 14. Serum magnesium concentrations during study period in control and study groups. (FU1 = 6 weeks post discharge; FU2 = 12 weeks post discharge; FU3 = 18 weeks post discharge).



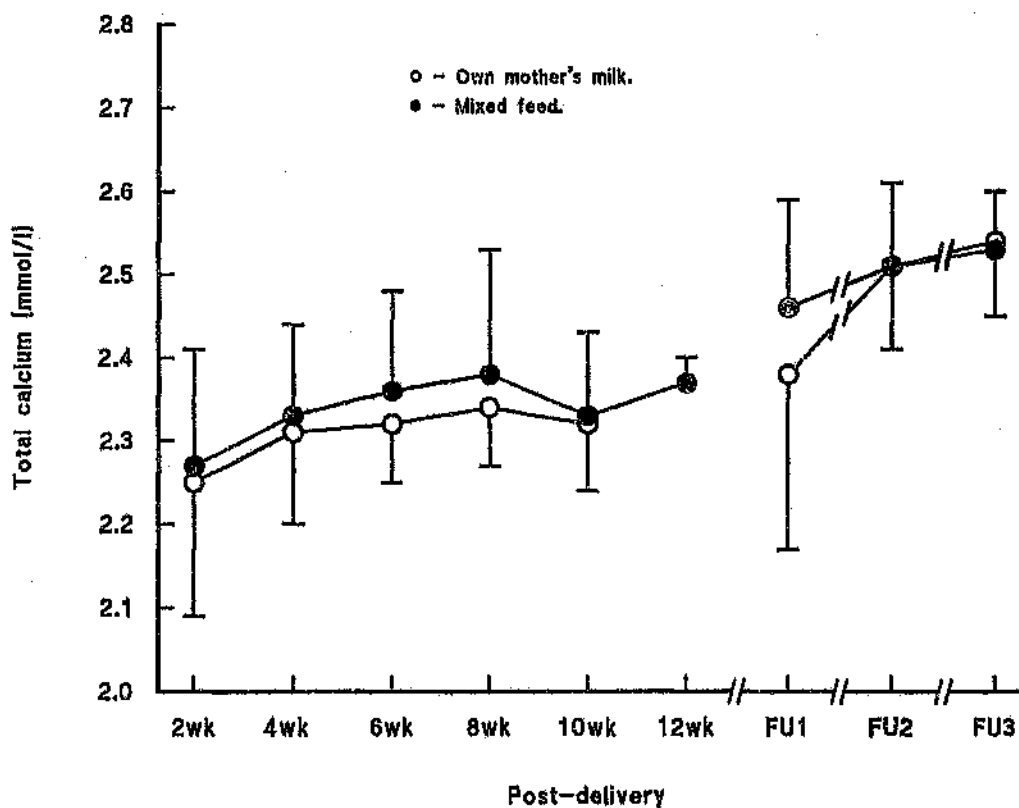


Figure 15. Serum calcium (total) concentrations during the study in control and study groups. (FU1 = 6 weeks post discharge; FU2 = 12 weeks post discharge; FU3 = 18 weeks post discharge).

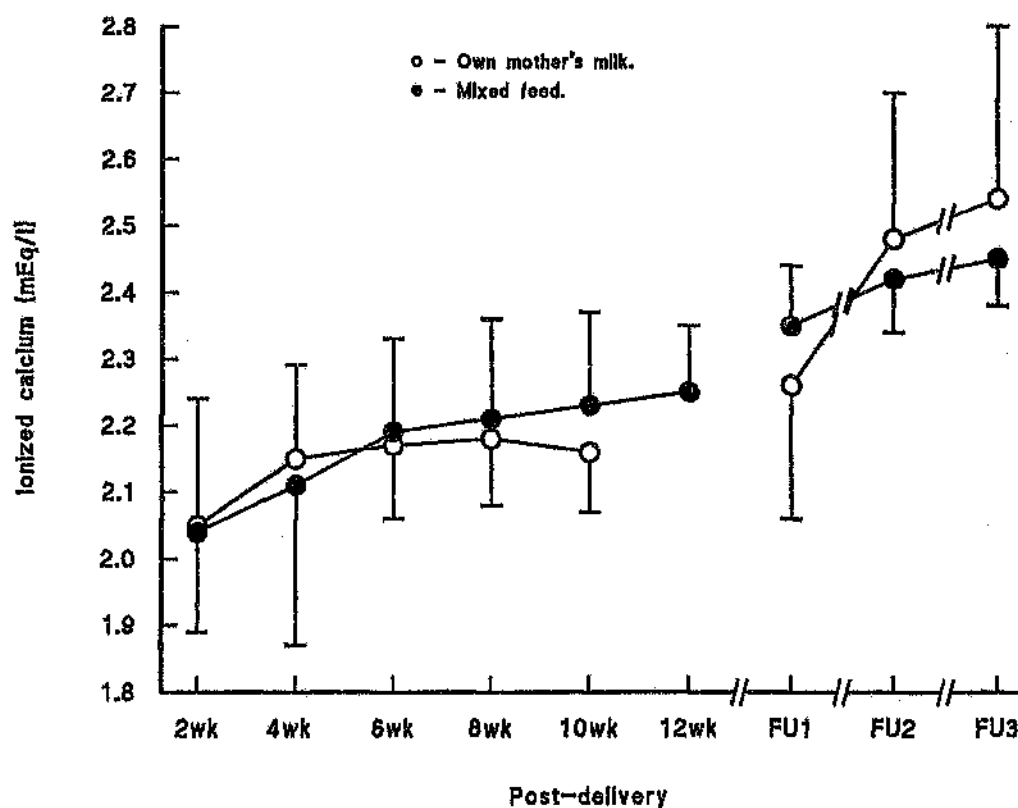


Figure 16. Serum calcium (ionized) concentrations during the study in control and study groups. (FU1 = 6 weeks post discharge; FU2 = 12 weeks post discharge; FU3 = 18 weeks post discharge).

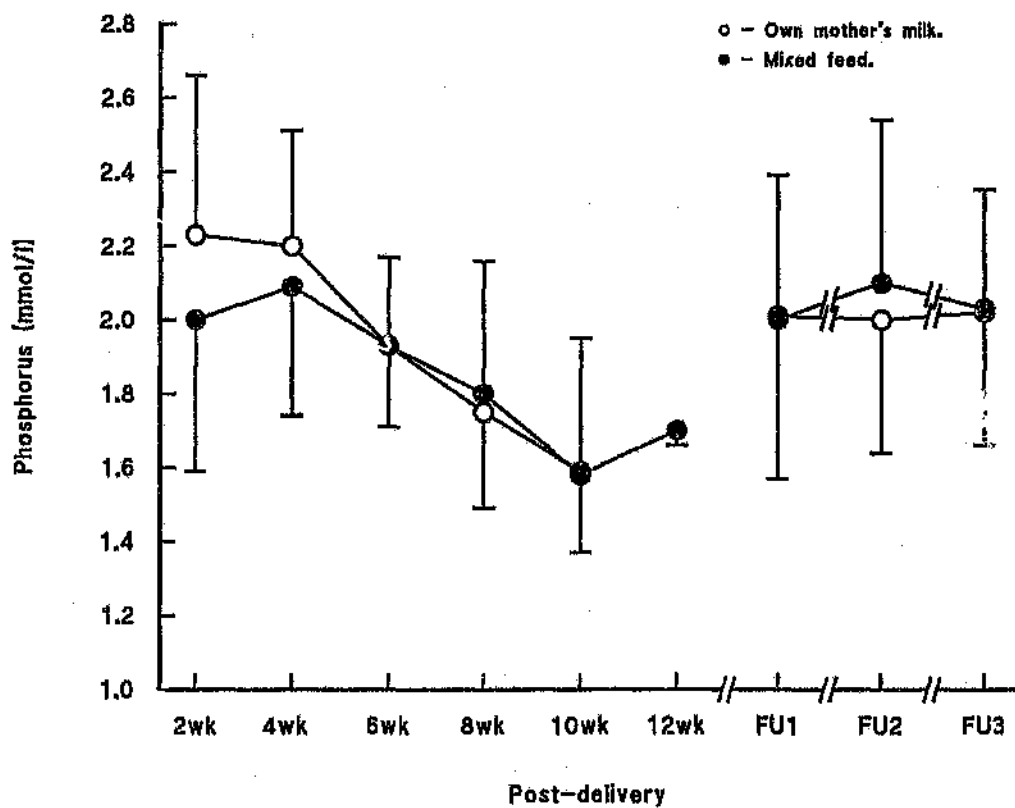


Figure 17. Serum phosphorus concentrations during the study in control and study groups. (FU1 = 6 weeks post discharge; FU2 = 12 weeks post discharge; FU3 = 18 weeks post discharge).

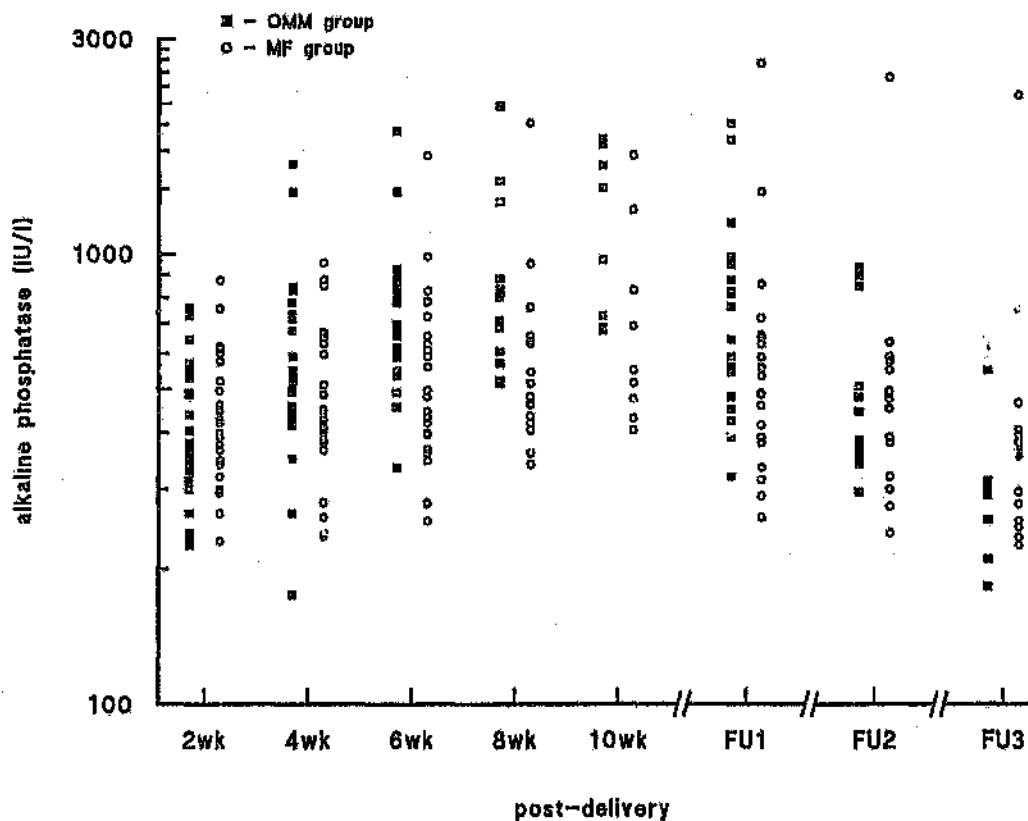


Figure 18. Serum alkaline phosphatase levels in control and study groups during the study period. (FU1 = 6 weeks post discharge; FU2 = 12 weeks post discharge; FU3 = 18 weeks post discharge).

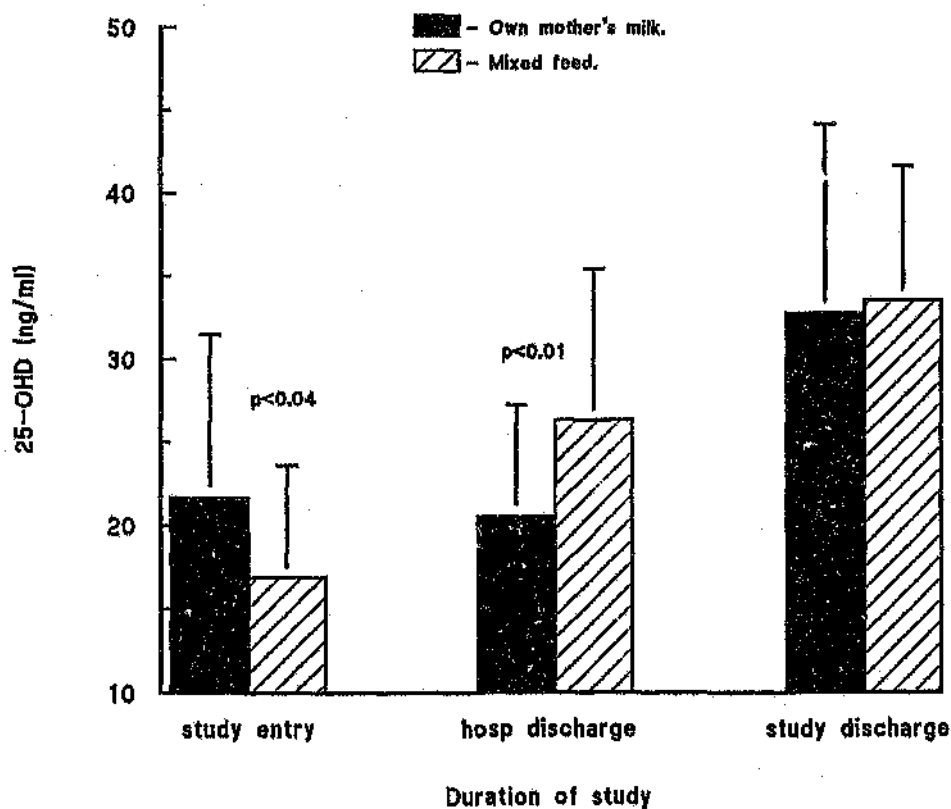


Figure 19. Serum 25-hydroxyvitamin D levels in control and study groups during the study.

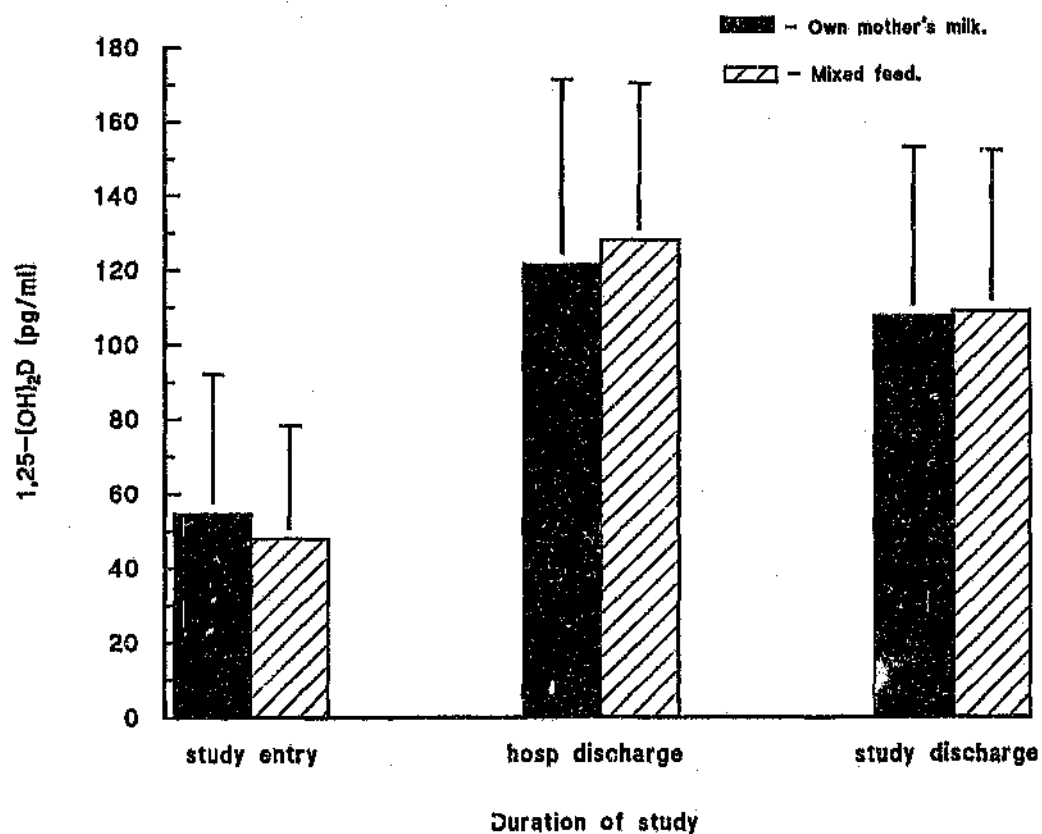


Figure 20. Serum 1,25-dihydroxyvitamin D levels in control and study groups during the study.

Combining the data from the three time points (i.e. at two weeks of age, at hospital discharge and at final follow-up), a negative correlation was found between  $1,25-(\text{OH})_2\text{D}$  and phosphate in both groups, although it only reached statistical significance in the study group ( $r=0.25$ ,  $p=0.026$ ) (Figures 21 and 22). A similar significant negative correlation was found between alkaline phosphatase and serum phosphate in both the study and control groups ( $r=0.43$ ,  $p<0.01$  and  $r=-0.51$ ,  $p<0.01$  respectively) (Figures 23 and 24). There was no correlation between serum alkaline phosphatase levels and serum ionized calcium in either group (OMM group  $r=-0.05$ ,  $p=0.68$ ; MF group  $r=0.004$ ,  $p=0.97$ ). There was a positive correlation between  $1,25-(\text{OH})_2\text{D}$  and serum ionized calcium (OMM group  $r=0.28$ ,  $p=0.03$ ; MF group  $r=0.37$ ,  $p<0.01$ ). A positive correlation was found between serum alkaline phosphatase levels and  $1,25(\text{OH})_2\text{D}$ , the correlation reaching significance in the study group ( $r=0.25$ ,  $p=0.026$ ) (Figures 25 and 26). There was no correlation between serum  $1,25-(\text{OH})_2\text{D}$  and 25-OHD levels in the own mother's milk group (Figure 27), however, in the mixed feed group a significant positive correlation was found  $1,25-(\text{OH})_2\text{D}$  and 25-OHD when assessed over the entire duration of the study period (Figure 31). When the data was divided into the specific time intervals, a significant correlation was only obtained at the time of discharge from hospital ( $r=0.4$ ,  $p=0.03$ ) (Figures 32-34).

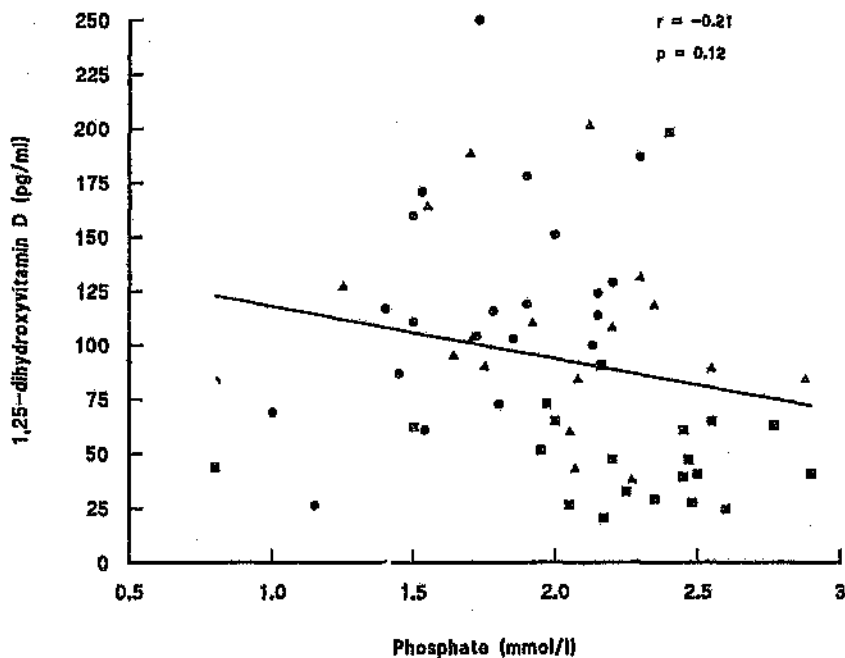


Figure 21. Serum 1,25-dihydroxyvitamin D versus phosphate in the own mother's milk group.

(■ = study entry; ● = hosp discharge; ▲ = study discharge).

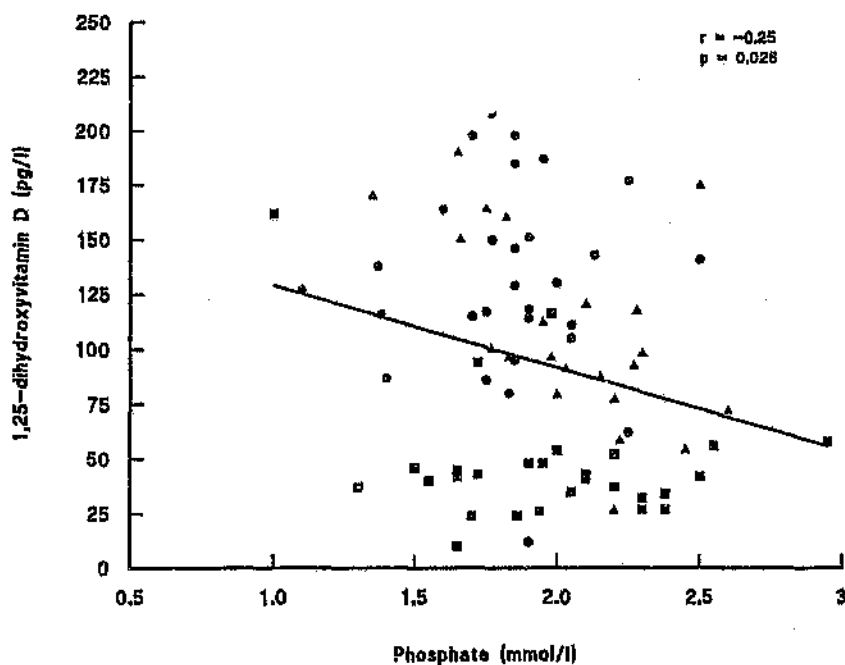


Figure 22. Serum 1,25-dihydroxyvitamin D versus phosphate in mixed feed group.

(■ = study entry; ● = hosp discharge; ▲ = study discharge).



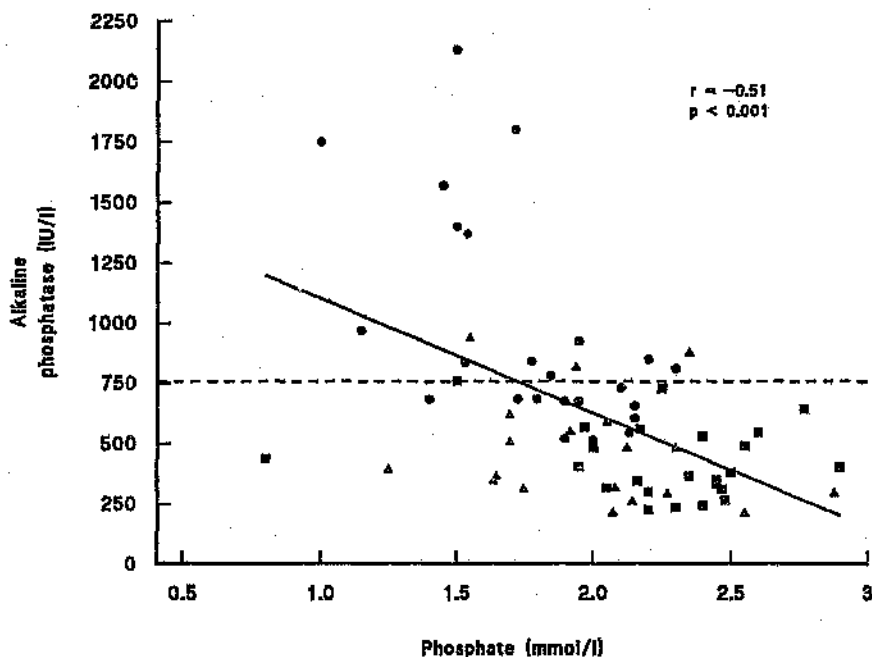


Figure 23. Serum alkaline phosphatase levels versus phosphate in own mother's milk group. (■ = study entry; ● = hosp discharge; ▲ = study discharge; --- cut-off pt above which alkaline phosphatase highly indicative of rickets).

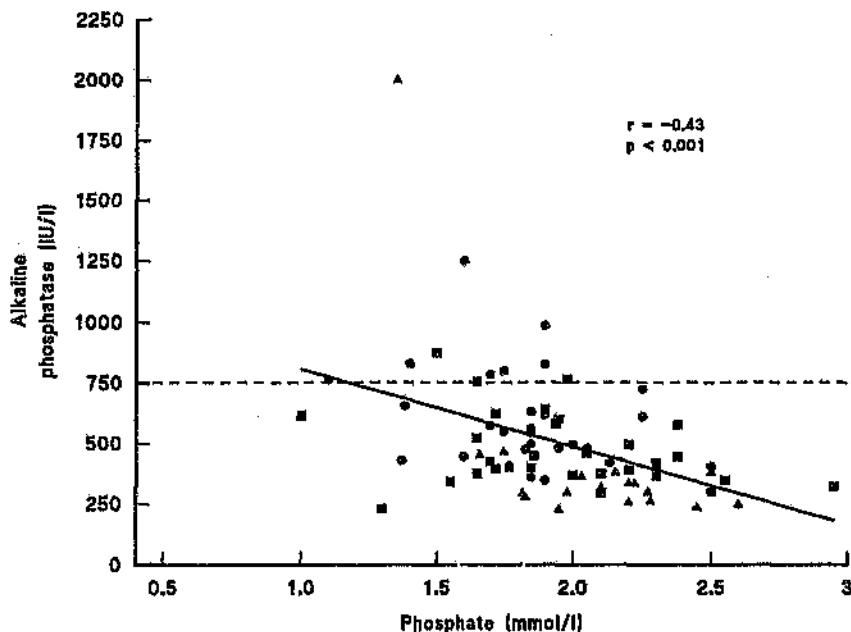


Figure 24. Serum alkaline phosphatase versus phosphate in mixed feed group. (■ = study entry; ● = hosp discharge; ▲ = study discharge; --- cut-off above which alkaline phosphatase indicative of rickets).

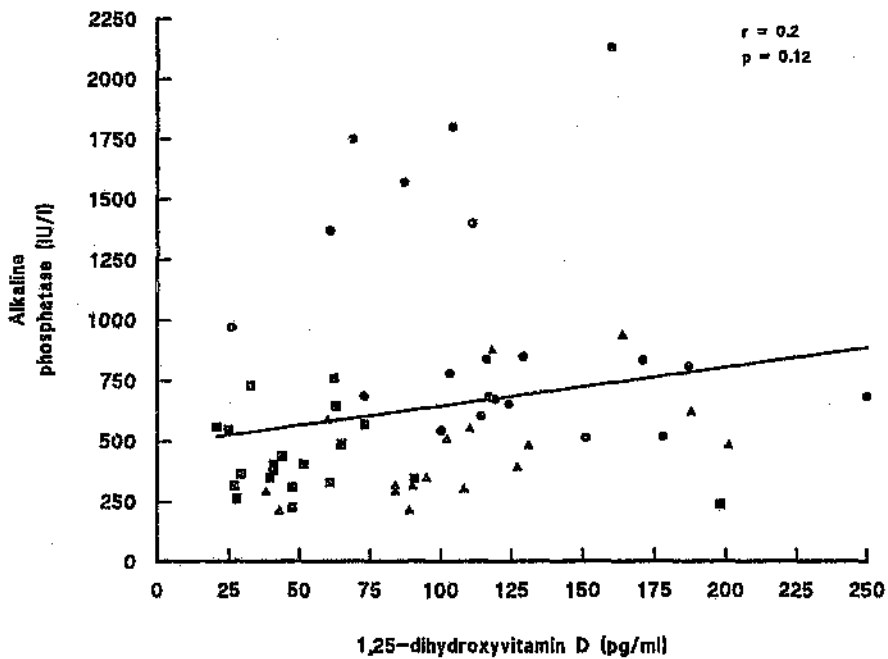


Figure 25. Serum alkaline phosphatase versus 1,25-dihydroxyvitamin D  
in own mother's milk group.  
(■ = study entry; ● = hosp discharge; ▲ = study discharge).

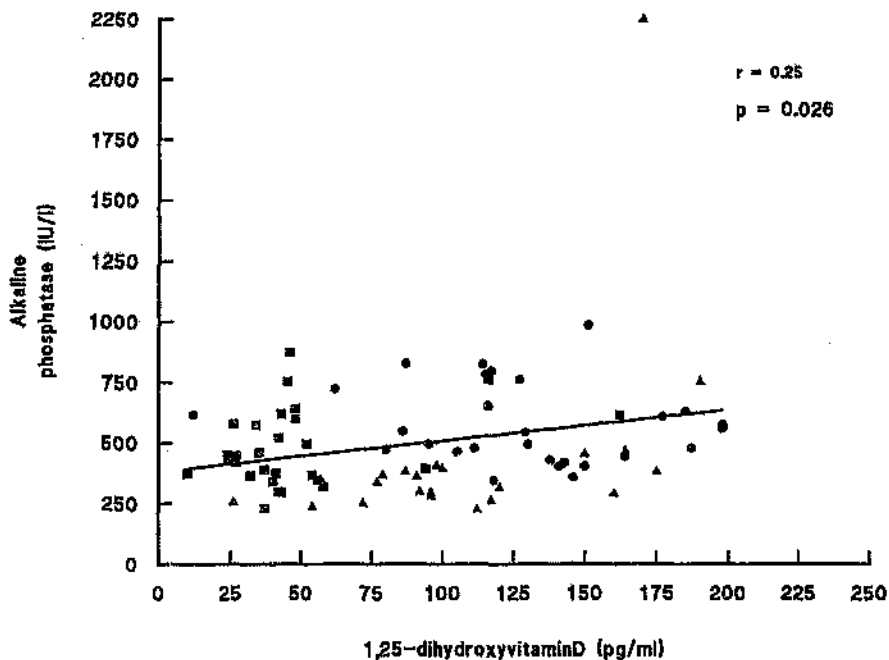


Figure 26. Serum alkaline phosphatase versus 1,25-dihydroxyvitamin D  
in mixed feed group.  
(■ = study entry; ● = hosp discharge; ▲ = study discharge).

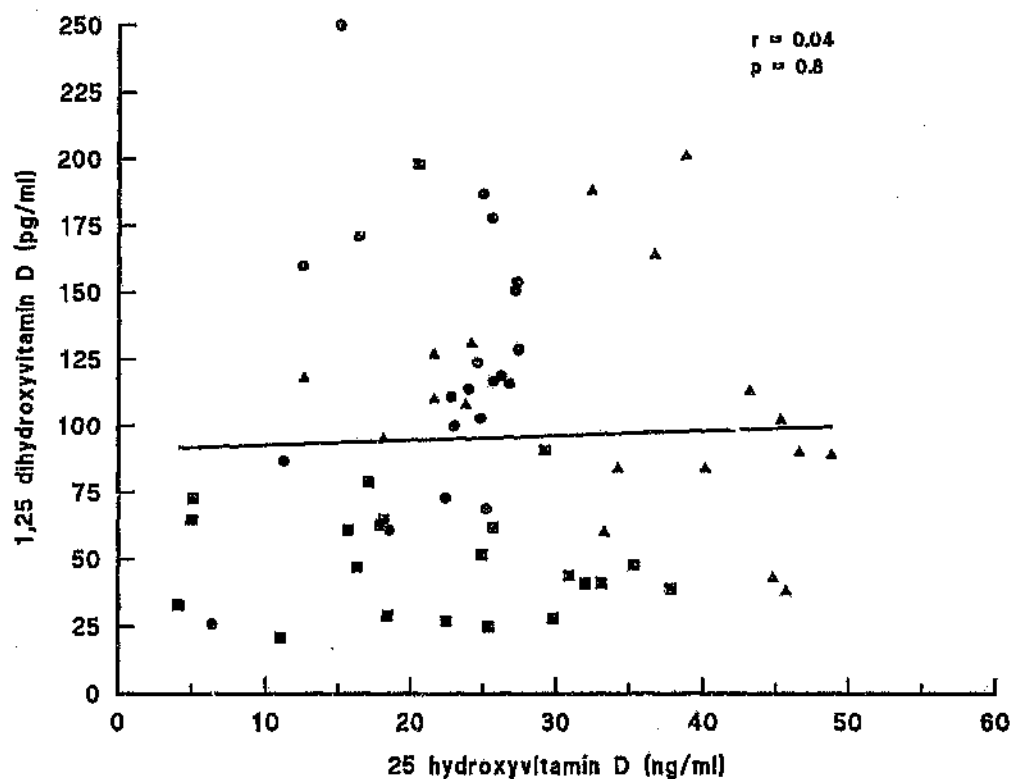


Figure 27. Serum 1,25-dihydroxyvitamin D versus 25-hydroxyvitamin D in the own mother's milk group.  
( $\square$  = study entry;  $\bullet$  = hosp discharge;  $\triangle$  = study discharge).

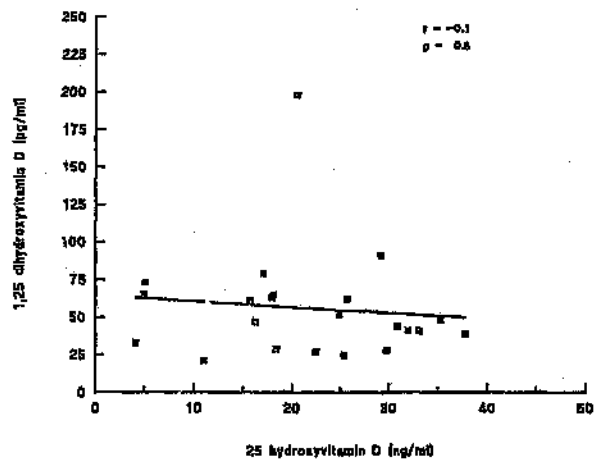


Figure 28. Serum 1,25 dihydroxyvitamin D versus 25 hydroxyvitamin D in own mother's milk group on entry into study.

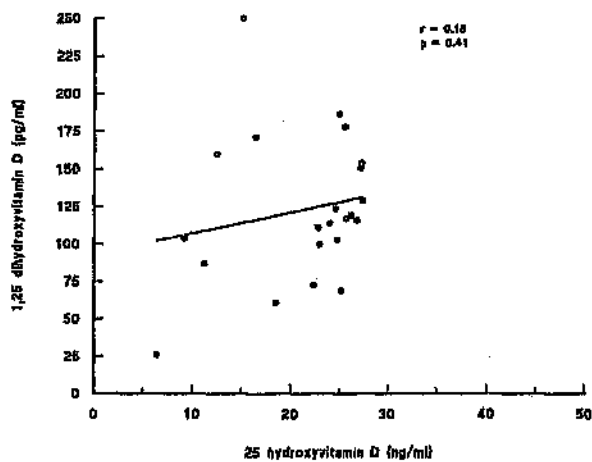


Figure 29. Serum 1,25 dihydroxyvitamin D versus 25 hydroxyvitamin D in own mother's milk group on discharge from hospital.

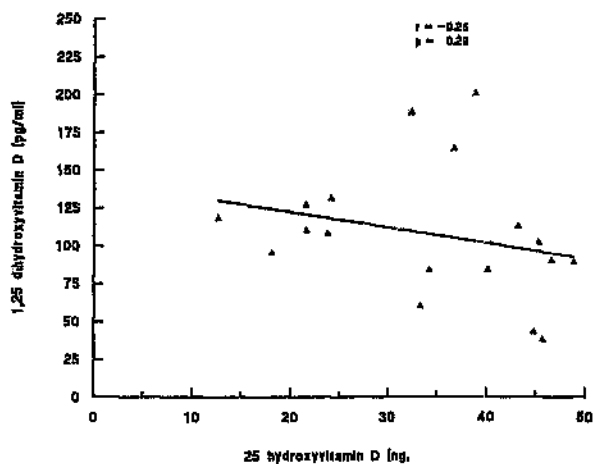


Figure 30. Serum 1,25 dihydroxyvitamin D versus 25 hydroxyvitamin D in own mother's milk group on discharge from study.

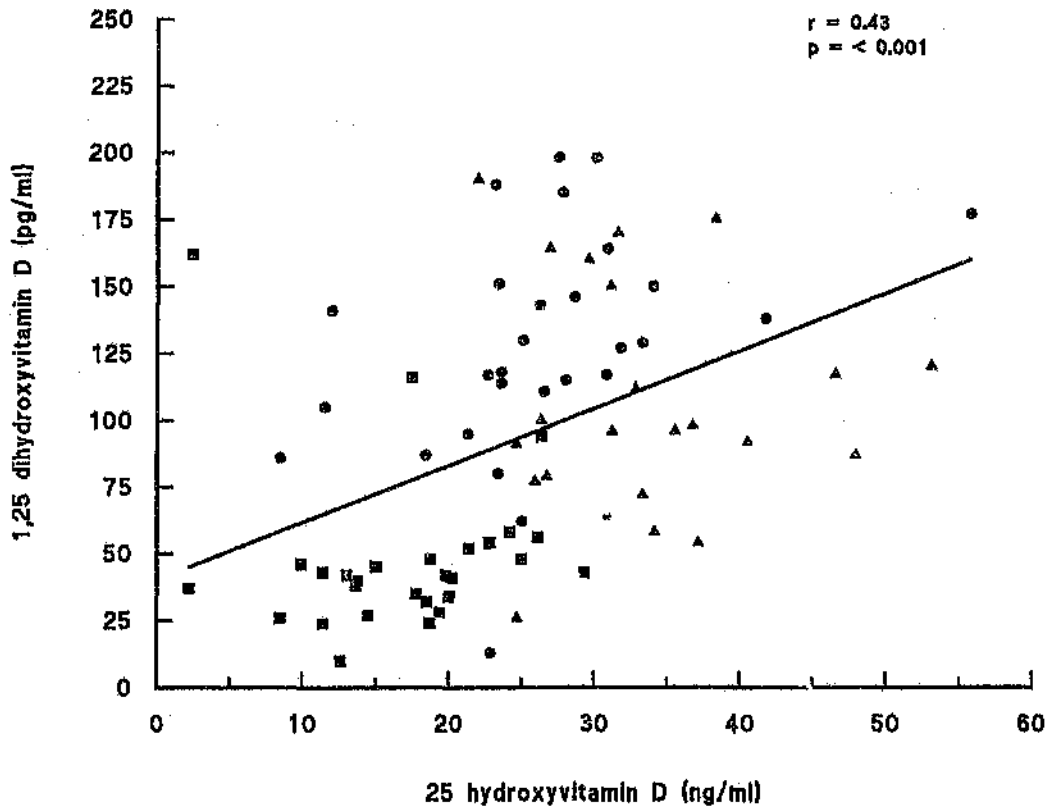


Figure 31. Serum 1,25-dihydroxyvitamin D versus 25-hydroxyvitamin D in the mixed feed group.

(■ = study entry; ● = hosp discharge; ▲ = study discharge).

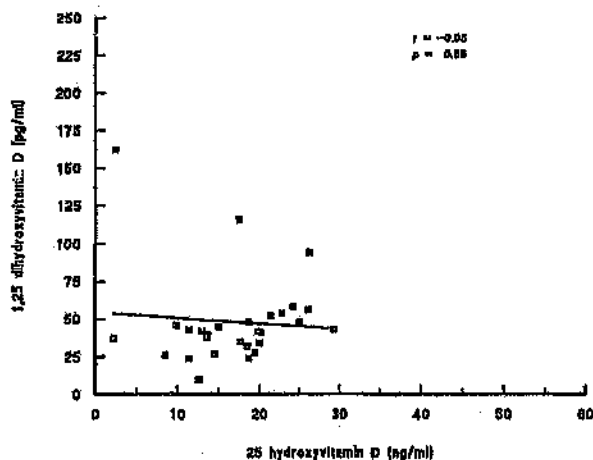


Figure 32. Serum 1,25 dihydroxyvitamin D versus 25 hydroxyvitamin D in mixed feed group on entry into study.

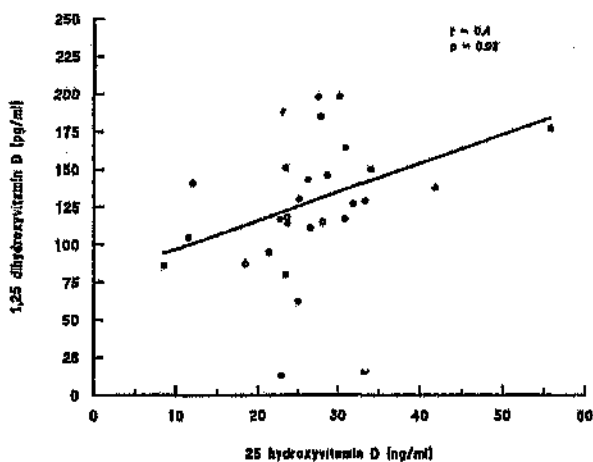


Figure 33. Serum 1,25 dihydroxyvitamin D versus 25 hydroxyvitamin D in mixed feed group on discharge from hospital.

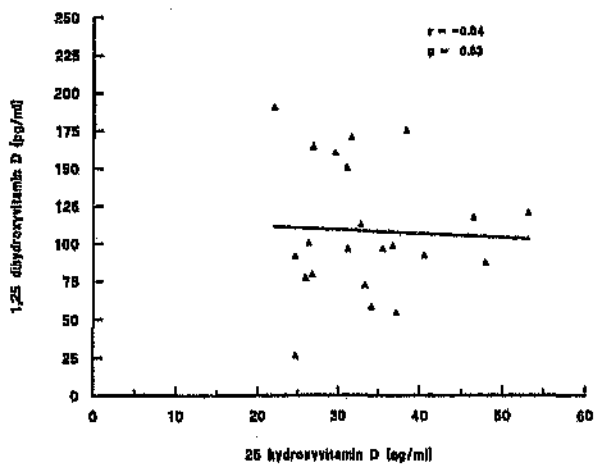


Figure 34. Serum 1,25 dihydroxyvitamin D versus 25 hydroxyvitamin D in mixed feed group on discharge from study.

### 3.5. Radiological findings

Radiological findings in both groups on discharge from hospital and at the final follow-up visit are summarized in Table 12.

**TABLE 12.** Prevalence of radiological findings in control and study groups on discharge from hospital and at final follow-up visit. (OMM = own mother's milk; MF = mixed feed).

	OMM		MF	
	Discharge	Follow-up	Discharge	Follow-up
Periosteal reaction	9/23 39%	2/15 13%	11/26 42%	0/18 0%
Osteopenia	6/23 26%	4/15 27%	8/26 31%	5/18 28%
Rickets	3/23 13%	1/15 7%	0/26 0%	1/18 6%

There were no statistically significant differences in radiological findings in the control and study groups. Periosteal reactions were noted in just over one third of patients at the time of discharge from hospital. The prevalence had dropped markedly by the time radiographs were repeated at the last follow-up visit. Those infants with periosteal reactions tended to have higher alkaline phosphatase levels ( $822 \pm 432\text{IU/l}$ ) than those without periosteal reactions ( $677 \pm 242\text{IU/l}$ ;  $t=1.31$ ,  $p=0.098$ ). The prevalence of osteopenia did not change significantly between the time of discharge from hospital to the final follow-up visit. There was no difference in alkaline phosphatase values

in infants with or without evidence of osteopenia ( $734 \pm 386\text{IU/l}$  and  $781 \pm 256\text{IU/l}$  respectively;  $t=0.41$ ,  $p=0.34$ ). Radiological rickets was an infrequent finding, despite the elevation of alkaline phosphatase values in the majority of infants at hospital discharge. In all cases, the severity of rickets was mild with only minimal loss of the metaphyseal plate and slight splaying and irregularity of the metaphysis. Although only five infants had radiological rickets, alkaline phosphatase levels were significantly higher in this group than in those infants without rickets ( $1153 \pm 668\text{IU/l}$  and  $709 \pm 286\text{IU/l}$  respectively;  $t=2.73$ ,  $p=0.005$ ).



## CHAPTER 4

### DISCUSSION

Metabolic bone disease is a well recognised problem in premature infants and in particular in VLBW infants fed breast-milk (42, 61). Radiologic rickets is, however, an uncommon finding, although osteopenia and biochemical evidence of MBD (hypophosphataemia and elevated alkaline phosphatase levels) are frequently noted in the first three months of life (1). The premature infant, especially the VLBW has increased mineral requirements (8). It is generally accepted in first world countries that VLBW infants should be fed premature milk formula which has been specifically modified to cater for these needs (42). However, at Baragwanath Hospital, which serves mainly the disadvantaged community of Soweto, the Neonatal Unit has a policy of encouraging breast-feeding where possible. The use of a premature milk formula as the sole feed for VLBW infants would reduce the prevalence of breast-feeding in this group of infants, which might adversely affect the long-term survival of these infants once they are discharged from hospital. The prevalence of MBD and altered mineral homeostasis is unknown in the VLBW population at Baragwanath Hospital. This study was, therefore, designed to assess the prevalence and to determine the effect of supplementing breast-milk with a premature infant formula. In this study, two groups of VLBW infants (one fed own mother's milk and the other fed a combination of premature formula and breast-milk)

born at Baragwanath Hospital were monitored for differences in growth, and biochemical and radiological development of metabolic bone disease. By combining the premature formula with breast-milk, the increased mineral and energy content of the premature formula is effectively diluted. This could explain many of the similarities in results between the two groups, which might otherwise have been more evident.

The high neonatal mortality rate in the immediate postnatal period occurred in mainly AGA neonates weighing less than 1000g at birth with severe hyaline membrane disease. The survivors that were subsequently enrolled in the study were mainly small-for-gestational age infants who did not have the problems of extreme prematurity.

Anthropometric growth parameters of weight, height and skull circumference followed similar patterns in the control and study groups. The energy content of the two feeds was similar (650kcal/l in OMM compared with 680kcal/l in the MF group), therefore this finding was not unexpected. The growth patterns also matched acceptable standards of growth for premature infants (80). Although the infants fed breast-milk had significantly lower serum albumin levels in hospital, reflecting the lower protein content of breast-milk, this did not affect growth. With regard to growth in this group of infants, it is therefore, acceptable to continue with current feeding practices at Baragwanath Hospital which aims at

encouraging and establishing breast-feeding in all newborns at the hospital.

Hillman et al (25) have defined four factors which appear to be important in mineral homeostasis in the VLBW infant. These are birth weight, mineral availability, serum 25-OHD concentrations (reflecting vitamin D status) and post-conceptional age.

Calcium and phosphorus intakes in both the control and study groups were suboptimal (29). The highest calcium and phosphorus intakes were attained in the study group (95mg/kg/day calcium and 56mg/kg/day phosphorus) compared with recommended intakes of 200mg/kg/day calcium and 100mg/kg/day phosphorus (72). Although the control group had significantly lower intakes of both minerals, this was not reflected in the serum calcium or phosphorus concentrations which were similar in both groups. Ionized serum calcium values were also similar in both groups. Normal values for serum calcium and phosphate in the VLBW infant have not been definitively determined. Concentrations of these minerals increased in all infants in this study following discharge suggesting that the in hospital levels were below normal. This fact in conjunction with the documented suboptimal mineral intake would suggest that the infants in both control and study groups were deficient in calcium and phosphorus.

In this study, as in studies by Hillman et al (25) and Brooke et al (1), radiologic rickets was an infrequent finding, but periosteal reactions and osteopenia as well as raised alkaline phosphatase levels occurred frequently suggestive of the presence of metabolic bone disease. Alkaline phosphatase is an indicator of osteoblastic activity and also bone turnover and levels five to six fold above normal reflect impaired bone mineralization and MBD (44, 49). In this study, alkaline phosphatase levels were significantly higher in those infants with radiological rickets and were also higher, although not significantly so, in those infants with periosteal reactions. These findings are suggestive of biochemical and radiological evidence of MBD, although they must be interpreted with reservation in view of the small number of infants affected and the wide standard deviation. The diagnosis of osteopenia is a subjective assessment and observer dependent and is, therefore, not as reliable a sign of MBD as are periosteal reactions and frank rickets. The failure to demonstrate a rise in alkaline phosphatase in association with osteopenia is thus not unexpected. Alkaline phosphatase levels rose simultaneously with a drop in serum phosphate suggesting the presence of MBD and possible phosphate deficiency. The increase in alkaline phosphatase levels and the negative correlation of alkaline phosphatase with serum phosphate that were found in the both groups are also suggestive of mineral deficiency.

It is suggested that these biochemical abnormalities are secondary to phosphate insufficiency (63). The typical biochemical abnormalities that occur in phosphorus deficiency are hypophosphataemia, hypophosphaturia and hypercalcaemia (63). The low phosphorus content leads to a decrease in serum phosphorus which has a direct impairment on bone mineralization. The decrease in serum phosphate concentration also has an indirect effect on bone mineralization by its stimulation of 1,25-dihydroxyvitamin D production by the kidney, which in turn leads to increased bone resorption and bone turnover resulting in increased alkaline phosphatase values. The significant increase in 1,25-dihydroxyvitamin D levels in both groups prior to discharge from hospital is suggestive evidence of stimulation of 1,25(OH)<sub>2</sub>D production by mineral (calcium and/or phosphorus) insufficiency. Several correlations found in the present study in both groups of infants support the hypothesis of primary phosphate deficiency. The negative correlation between serum 1,25-(OH)<sub>2</sub>D and serum phosphate which was significant in the mixed feed group and approached significance in the breast-fed group is indicative of stimulation of 1,25-(OH)<sub>2</sub>D production by low serum phosphate. This also confirms previous work demonstrating that renal hydroxylation of 25-OHD to 1,25-(OH)<sub>2</sub>D is adequate (30, 33). The positive correlation found between alkaline phosphatase and 1,25(OH)<sub>2</sub>D in both groups is suggestive of increased bone turn-over stimulated by 1,25(OH)<sub>2</sub>D in an attempt to maintain serum phosphate

concentrations within normal, thereby completing the cycle.

The lack of correlation between serum calcium and alkaline phosphatase suggests that calcium deficiency did not play a role in the development of metabolic bone disease in the control or study groups in this study. The finding of a significant positive correlation between serum calcium and  $1,25(\text{OH})_2\text{D}$  suggests a relative calcium excess in the face of phosphate deficiency adding further support to the hypothesis that primary phosphate deficiency is the major aetiological factor in altered mineral homeostasis.

Parathyroid hormone concentrations were not measured in this study and, therefore, could not be correlated with changes in vitamin D metabolism.

Numerous studies have confirmed that the supplementation of calcium and phosphorus to the feeds of VLBW infants may prevent the development of MBD. It was not possible to fully assess this aspect in this study as neither feed supplied the required amount of calcium or phosphorus in order to achieve in utero accretion rates. The concentration of minerals supplied in the modified premature formula was negated in this study by its dilution with own mother's milk. However, the study group did have lower alkaline phosphatase values than the control group, suggesting that the additional calcium and phosphorus did reduce the severity of the

perturbations in mineral homeostasis.

As reported by Hillman et al (25), serum concentrations of 25-hydroxyvitamin D are also a determining factor in the development of metabolic bone disease. 25-OHD is the major circulating form of vitamin D and is thus a reflection of vitamin D status (5, 6). The premature infant has increased vitamin D requirements (800IU/day), although no definitive evidence of immaturity in the metabolic pathway of vitamin D has been documented (30-33). Immaturity of the hepatic hydroxylation system has been documented, but is of doubtful significance. Abnormalities of vitamin D metabolism probably do not play a major role in the pathogenesis of MBD provided that an adequate vitamin D intake is supplied. All infants in this study received 800IU vitamin D from two weeks of age. The mixed feed group in this study had a significant increase in serum 25-OHD levels prior to discharge from hospital, while in the breast-milk fed group in whom the increase only occurred following hospital discharge. This might be accounted for by the slightly higher vitamin D intake in the mixed feed group (approximately 100IU/day) due to that contained in the premature formula. It is possible that this vitamin D is also available in a more readily absorbable form. It is unlikely that inadequate hepatic hydroxylation played a role as this would have been evident in both groups. The lack of correlation between  $1,25(\text{OH})_2\text{D}$  and 25-OHD levels in both groups at birth and on discharge

from the study is in keeping with vitamin D repletion. The tendency towards a positive correlation between these two parameters prior to hospital discharge may suggest a relative vitamin D deficiency despite adequate intake, although the number of cases in each group at this time is too small for definitive comment.

Decreasing gestational age and birth-weight (25, 37), and systemic disease and acidosis have also been shown to be predisposing factors in the development of MBD. The majority of infants finally enrolled and completing this study were small-for-gestational age who had minimal number of post-natal complications considering their birth-weight. The AGA infants weighing under 1000g at birth and in whom a higher prevalence of MBD would have been expected, were self excluding by virtue of Baragwanath Hospital policy of not ventilating neonates born less than 1000g. These factors might explain the relatively low prevalence of radiological rickets in the population studied.



## CONCLUSIONS

1. Overt rickets is not a major problem in very low-birth-weight infants born at Baragwanath Hospital, although biochemical evidence as assessed by raised serum alkaline phosphatase levels, and radiological evidence as assessed by periosteal reactions and osteopenia occurs frequently.
2. Hypophosphataemia, normocalcaemia and raised 1,25-dihydroxyvitamin D levels support the hypothesis of phosphate deficiency as a primary cause of metabolic bone disease.
3. The mixing of breast-milk with a specialized premature milk formula (Alprem) has no major advantage on weight gain and growth, or on the development of metabolic bone disease in very low-birth-weight infants.
4. It is recommended that the use of own mother's milk be encouraged in the light of these results and that current feeding practices of very low-birth-weight infants at Baragwanath Hospital be continued with the aim of establishing breast-feeding.
5. Biochemical monitoring, especially that of alkaline phosphatase, should be performed in very low-birth-weight infants. Those with elevated levels greater than 750IU/l should be further investigated and supplemented with calcium and phosphate if necessary.

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**Name of thesis:**Rickets in very low-birth-weight infants born at Baragwanath Hospital

***PUBLISHER:***

University of the Witwatersrand, Johannesburg

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